An Investigation Of Natural Hybridization Between Jack Pine (*Pinus banksiana*) And Lodgepole Pine (*Pinus contorta* var. *latifolia*) In Northern British Columbia

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Abstract

This study was conducted in order to find evidence of hybridization between jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta* var. *latifolia*) in Northeast British Columbia (BC) through genetic identification of paternity and maternity of each sample, while relating differences in morphology, wood and fibre traits, and chemical makeup to genetic identity. Adding to this, an attempt was made to determine if wood and fibre traits can be used as distinguishing features between jack pine, lodgepole pine, and their hybrids, and to determine if a "chemical extractive footprint" can be used to differentiate between species groups.

Thirty samples of pure lodgepole pine, 30 of pure jack pine, and 30 potential hybrid samples were collected from the Prince George area of BC, the Smoky Lake area of Alberta, and the Fort Nelson region of BC, respectively. Data was analyzed by comparing characteristics in order to establish trends and interactions between characteristics, site conditions, and differences among samples.

Needles from foliar regions and inner cambial layers were harvested for DNA analysis. Chloroplast DNA and mitochondrial DNA were used to determine hybridization between lodgepole pine and jack pine by restriction fragment length polymorphism (RFLP). Gross morphological characteristics of each tree were measured from collected cone and needle samples, including, cone length and orientation, and needle length and position. Tree height and diameter at breast height (DBH) were recorded at time of sampling. Two 10 mm cores (bark to bark) were taken from each tree and analyzed for fibre length and coarseness through Fibre Quality Analysis, and for microfibril angle, basic density, earlywood : latewood ratios, modulus of elasticity (MOE), and cell wall configuration using SilviScan technology. Chemical extractive makeup of each sample was determined using gas chromatography- mass spectroscopy.

Based on the genetic evidence, 16 out of 24 samples from the introgression zone clearly show hybridization, with lodgepole pine maternity and jack pine paternity, occurring in the Fort Nelson region. Morphological characteristics that have been identified to distinguish among species groups include: needle V width/length, cone angle of attachment, cone curvature, cone length, and DBH/age. Cell and fibre traits that best differentiated between pure jack pine, lodgepole pine, and hybrids were moisture content (MC), MOE, cell wall thickness, and fibre coarseness, while only MC and MOE displayed hybrid intermediacy clearly.

Preliminary chemical analysis of the samples indicates that quantitative variability does exist between jack pine, lodgepole pine, and hybrid sample wood extractives; however, a clear "chemical extractive footprint" can not be determined. Further manipulation of chromatographs and more extensive investigation of mass spectra are required.

Revealing how natural hybrids are different from pure species, and what this means to the scientific and industrial communities as well as forest managers is of primary importance. Supportive evidence of hybridization and introgression in the Fort Nelson region of British Columbia will provide information for proper management of forests in this region, with regard to silvicultural practices and tree breeding, and aid in optimization of processing and manufacturing to improve wood and product quality.

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Dedication:

I would like to dedicate this very long piece of work, and with it more than 2 years of time, energy, and life experience, to my husband, Keith, my mom, June, and my dad, Joe.
Keith, thank you for encouraging and pushing me to strive for my goals and for providing me with an example of work ethic that is untouched by anyone else I know.
Mom and dad, thank you for always supporting me and allowing me to believe that I could meet any challenge that comes my way.
I love you all very much!

Chapter 1: Introduction

1.1 Introgression and Hybridization

Introgressive hybridization can be defined as "...the infiltration of genes of one species into another through repeated backcrossing of hybrids to one or both parental species." (Wheeler and Guries, 1987). Introgression can be seen as a step in evolution; increasing genetic information and providing new gene interactions (Wheeler and Guries, 1987). Another definition describes hybridization is the "...interbreeding of two populations, or groups of populations, which are distinguishable on the basis of one or more characters..."(Woodruff, 1973). Woodruff (1973) furthers this definition by saying that hybridization produces individual hybrids that are recognized by one or more taxonomic features. Even with these definitions in mind, classifying hybrids using the taxonomy that exists today can be challenging. Hybrid pines that are under investigation in this study will fall into the same taxonomic "grey area". If a lodgepole pine (*Pinus contorta* var. *latifolia*) x jack pine (*Pinus banksiana*) hybrid is found to exist not only morphologically, but genetically and based on wood, fibre, and chemistry traits, then a system of classification must be established in order to properly identify these hybrid individuals.

1.1.1 Characterization of Hybrid Zones

The definition of species, "...as a set of populations delimited by genetic barriers to gene exchange..."; or populations that naturally interbreed and are reproductively isolated from other populations, is contrasted by the existence of hybridization (Barton and Hewitt, 1985).

In order to interpret hybrid zones in terms of common taxonomy, the zones can be characterized as areas where genetic information is exchanged between two different species

populations to create a range or gradient of genetic forms between the two species. This genetic gradient is referred to as a "cline" (Barton and Hewitt, 1985) in order to distinguish gradient genotypes and phenotypes from hybrids, which are often seen as only one recombined phenotype or genotype of the parental species. Clines are not limited to hybrids of a particular form or morphology; these 'gradient hybrid zones' move with reproduction and dispersal area of seed (Barton and Hewitt, 1985). Clines can be further described as either allopatric zones, where only the hybrid species is present in a narrow area between both parental species that do not meet, or as sympatric zones, where hybrids and parental species are located in the same area heterogeneously (Short, 1969). Sympatric zones can be further classified as parapatric, if the cline is quite narrow; see Figure 1 and Table 1 (Woodruff, 1973).

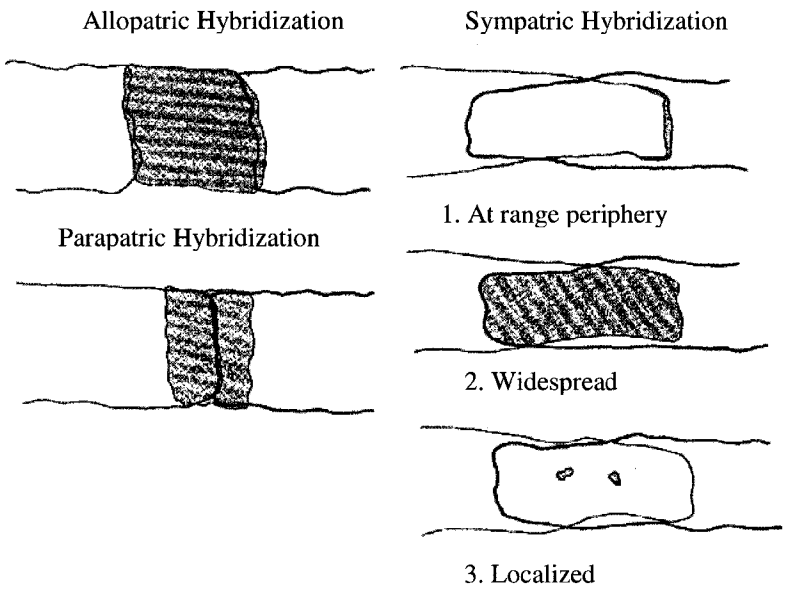

Figure 1: Schematic diagrams illustrating allopatric and parapatric hybrid zones, and three types of sympatric hybridization. Hybrids are indicated by shading, geographic ranges of parental species are outlined (from Woodruff, 1973).

Table 1: Suggested Classification for Hybrid Zones by Woodruff (1973).

Distribution of Parental Types	Distribution of Hybrids	Suggested Terminology
allopatric	between ranges of parental types	intergradation or allopatric hybridization
parapatric	adjacent to contact	parapatric hybridization
sympatric	associated with peripheral populations	peripheral sympatric hybridization
sympatric	localized	localized sympatric hybridization
sympatric	widespread	widespread sympatric hybridization

Clines are established when two distinct populations come into contact. Advantageous alleles will advance. Differences in fitness causes certain individuals to perpetuate better than others; two populations head towards an equilibrium, or hybrid population, that may be better evolved than the two original populations for the surrounding environment (Barton and Hewitt, 1985). It is likely that a gradient of hybridization, or cline, exists where any introgression zone is verified. Individual hybrids found within a cline may contain genetic information from each parent species in any percentage, and is not necessarily a generation 1 hybrid; lodgepole pine from one side, jack pine from the other. Therefore genetically identifying these hybrids is difficult and may not always be possible using the RFLP (restriction fragment length polymorphism) method that is used in this study.

New hybrids are formed within a cline from the flow of genetic information from individual to individual. Many factors contribute to the movement of a cline; gene flow does not always favor the fittest. The most influential factors are stand density and seed dispersal

rates although dominant alleles will spread more frequently than recessive (Barton and Hewitt, 1985).

Since jack pine stands are segregated into random patches across the landscape in the western reach of the jack pine range, hybrids or clines may be more infrequent. Variation among jack pine may be limited due to patchiness, and hybrid types may be less diverse than in lodgepole pine stands in Northern BC. Pine stands in BC are large and vast allowing for increased hybrid gradients and ease in cline movement over the landscape.

As genetic information flows between populations creating variety in pine species, the movement of alleles from generation to generation can meet certain physical or genetic barriers. Physical barriers include environmental changes that affect seed dispersal or density of a stand. Movement from an even-aged aged stand to an uneven-aged stand can also be a physical barrier to cline movement because not all trees would be at an age where they are able to reproduce. By the same token, cline movement from a single species stand to a multiple species stand could also act as a physical barrier; not all trees would be compatible for reproduction. Other physical barriers that affect hybrid zone movement or formation include climatic gradients, differences in vegetation, and differences in soil type. Human development and disturbance can also be seen as a physical barrier to gene flow, producing variation in hybrids to best suit the surrounding environment (Barton and Hewitt, 1985). Barton and Hewitt go on to suggest, however, that environmental gradients will not maintain a hybrid zone, or keep it in the same position. Genetic barriers include the fitness of individual trees; reproductive capability can be lowered by pathogen or pest infestation thereby impeding gene flow.

Since barriers to gene flow are random and gene flow is asymmetric, clines do not necessarily move in an even pattern (Barton and Hewitt, 1985). This accounts for the variability in hybrid locations. Jack pine x lodgepole pine hybrids, although approximately located in the introgression area where population ranges overlap, may be found outside of this zone, for example in an area northeast of Prince George, BC due to cline movement over time.

Due to the high amount of genetic recombination that is on going within a hybrid zone, a high percentage of rare alleles can be found. This characteristic is compounded by high mutation rates and somewhat relaxed selection in some cases (Barton and Hewitt, 1985). However, this would only apply to hybrid zones that have not reached equilibrium with surrounding species. Well-equilibrated populations would be more homogenous or allopatric in nature.

1.1.2 Evolutionary Significance

Species develop as an adaptive response to their environments as well as in concordance with the gene flow available to them. These responses allow some species to hybridize and evolve populations with greater fitness. These populations form hybrid zones that possess a balance between dispersal and selection, and allow a wider range of environments to be tolerated (Barton and Hewitt, 1985). Evolution of species involves the recombination of genetic information through reproduction. Various factors are known to modify reproductive processes, individuals within a population, or populations themselves. Modifiers arise due to mutations within a hybrid zone, and decreases in gene flow from outside of the zone. These modifications to a population are then able to gain proportional advantages over the gene pool and perpetuate within the area (Barton and Hewitt, 1985).

From an evolutionary standpoint, if a certain zone is modified and barriers exist so that the gene input from outside the zone is restricted and gene flow is somewhat limited to within the hybrid population, then the hybrids in question may potentially be referred to as a new species. Given that species are reproductively isolated from others and display unique and individual characteristics. We can say that it may be possible to distinguish this type of population as a new species or subspecies based on the possibility of hybrid isolation. The classification of the hybrid zone could also aid in the argument of the development of a new species (Woodruff, 1973). An allopatric population that is segregated from parental types and reproductively self-sustaining would be more likely to fit the definition of a separate species than a sympatric population.

Specifically identifying evolutionary steps within the jack pine and lodgepole pine populations is somewhat challenging. Conifer populations have a large history and tracking this information in order to establish exact relationships and interactions between these two closely related pines requires methods to quantify genetic distances and data from the fossil record. Nei (1973) established a method to measure genetic differences in any species and establish the "genetic distance" between two different populations. Genetic distance, expressed as "D", can be found by the following equation "where $J_{xy} = \sum x_i y_i$, $J_x = \sum x^2_i$, $J_y = \sum y^2_i$, and x_i and y_i denote the frequency of the *i*th allele in the population x and y respectively" (Danick and Yeh, 1983):

$$D = - \log_e [J_{xy}/(J_x J_y)^{1/2}] \qquad [1]$$

Danick and Yeh (1983) applied this directly to lodgepole and jack pine and found genetic distances using the Nei equation for Alberta populations using allozyme markers. They found that the genetic distance between the two species was 20 times greater than and interspecific distance. Therefore, variation within species is much less genetically identifiable than variation between jack pine and lodgepole pine. The fact that the genetic distance between jack pine and lodgepole pine is greater than the genetic distance between individuals within species, these genetic distance values being comparable to those observed in other organisms, supports that jack pine and lodgepole pine are two very distinct species. These are species that possess extensive variation in range, and that can not be misinterpreted as different variations of the same species from any point in history.

1.1.3 Hybridization vs. Divergence

An alternate hypothesis exists to counter the question of jack pine x lodgepole pine hybridization, namely divergence. The hypothesis states that the jack pine and lodgepole pine species are not presently hybridizing, or exchanging genetic information over time, but rather these species are diverging from one original species due to environmental adaptation and have been since the last ice age occurred approximately 18,000 years ago (Critchfield, 1985; Barton and Hewitt, 1985). Pollen from the fossil record supports the fact that a variation in species existed, at the time of the last ice age, which is not distinguishable as lodgepole pine or jack pine. This pollen can not tell us if this species is the primitive ancestor of the two species that now exist or an evolved hybrid of lodgepole and jack pine (Critchfield, 1985). To add to this, little historical evidence exists regarding hybrid zone formation, stability, or movement, but most have been fairly static for at least the last century (Barton and Hewitt, 1985). With this in mind, it is difficult to determine is lodgepole pine x

jack pine hybrids exist today due to historical divergence or hybridization, however, plenty of evidence exists to confirm genetic recombination in natural environments today.

1.1.4 References to Lodgepole x Jack Pine Hybrids

According to Zavarin et.al. (1969), lodgepole pine x jack pine hybrids "...are readily recognized in the field and have been verified by comparison with artificial [crosses of these species]..." (Wheeler and Guries, 1987). Wheeler and Guries (1987) go on to say that there is concordance between the morphological characteristics of lodgepole x jack pine hybrids and electrophoretic evidence collected, leading to the assumption that gene exchange does take place between these pine species.

Since morphology is variable within the same species, the reliability of purely characteristic data is subjective. However, jack pine species shows no variation in morphology west of Lake Nipigon, Ontario, until the potential introgression zone with lodgepole pine (Critchfield, 1985). Danick and Yeh (1983) observed that the variability within populations of jack pine and lodgepole pine in Alberta is approximately normal compared to populations of other conifers. Contrary to Barton and Hewitt (1985), Dancik and Yeh (1983) believe that high genetic variability exists in conifer populations due to "...lack of effective barriers to gene flow...". Other contributors to genetic variability are population size, breeding systems, and ecological amplitude. Distribution of jack pine x lodgepole pine hybrids in the Alberta introgression zone is uneven and allele groups are patchy. This genetic variability and population lay-out is most likely due to restricted seed dispersal; seed falling close to the maternal parent produces small patches of trees with allelic similarity. Therefore cross-pollinations in these types of populations can be said to occur

between "...trees closely related by decent..." or trees that are evolutionarily similar (Dancik and Yeh, 1983).

The suggestion of hybridization between these two species is a possibility using historical evidence from pine pollen dating back to the Pleistocene (~18,000 years ago). Pollen evidence suggests that lodgepole and jack pine may have been exchanging genes since late-postglacial times.

Hybrids have been noted, described, and studied in Central and Northern Alberta as well as in the Mackenzie District of the NWT (Critchfield, 1985). This study's attempt to describe and identify hybrids in northeastern BC will add further dimension to this known introgression zone.

Extractive chemistry has been another area of study to support the existence of lodgepole x jack pine hybrids. Monoterpene analysis has been suggested as a "...sensitive method for detecting introgression..." (Pollack and Dancik, 1985); extractive compounds are studied in Chapter 5 in order to establish a chemical footprint that may identify between lodgepole pine, jack pine and hybrids.

Genetically, hybrid characteristics can be identified through analysis of isozyme markers, restriction fragment length polymorphisms (RFLPs), chloroplast DNA (Kormutak et.al., 1993), mitochondrial DNA, allozyme variation, and random amplified polymorphic DNA (RAPD) markers. Out of these methods, RFLPs of both mtDNA and cpDNA were chosen for this study in order to give a well rounded description of the DNA inputs and due to their ease of interpretation.

1.1.5 Evidence of Jack Pine and Lodgepole Pine Hybridization

There is extensive evidence regarding morphological characteristics which suggests hybridization between lodgepole and jack pine. That is, there are trees that display a combination of morphological characteristics of both species. In a study conducted by Wheeler and Guries (1987), 4 putative hybrid populations of the 37 pine populations sampled were identified as hybrids due to cone and branch characteristics. Locations of these populations surround Blue River, White Court, Grande Prairie, and Wapiti, Alberta. The hybrid morphological characteristics interpreted in an index were correlated to gene frequency data to reveal that introgression in these areas was statistically significant (by least-squares regression and pairwise comparison; all correlation coefficients were greater than 0.98).

Jack pine populations of eastern Alberta were shown to have a significant level of influence from lodgepole pine populations by evidence of genetic variation at enzyme loci (Critchfield, 1985).

Other examples of pines found with morphological characteristics of lodgepole x jack pine hybrids were between Yellowstone Lake, Wyoming, and Banff, Alberta; these trees displayed cone angles significantly smaller and more variable than lodgepole pine, but not completely curved towards the branch as in jack pine (Critchfield, 1985).

Evidence of introgression taken from chemical constituents of tree extractives is supported by Pollack and Dancik (1985), who tested lodgepole and jack pine for levels of β-phellandrene and α-pinene. According to the study, β – phellandrene occurs in much higher concentrations in lodgepole pine then in jack pine, and α-pinene is found in much higher concentrations in jack pine than lodgepole pine. At sample sites Two Creeks, Rocky

Mountain House, and Twin Lakes, populations were found with moderate concentrations of both β-phellandrene and α-pinene. These results are supportive of hybridization between lodgepole pine and jack pine. It has been stated however that "...variations in the composition of wood resin are inconsistent and difficult to interpret" (Critchfield, 1985).

Putative lodgepole x jack pine hybrids from the introgression zone in central Alberta have been characterized by chloroplast DNA restriction fragments which differ from the parental species, and may suggest lodgepole pine paternity (Kormutak, et.al., 1993).

1.1.6 Experimentation with Cross-Pollination between Species

According to Critchfield (1985), lodgepole pine and jack pine can be very easily crossed. Crosses that are performed with lodgepole pine as the female parent result in approximately 30% germinal seeds. Performing cross-pollination experiments in a laboratory setting allows reproductive barriers that exist in nature to be overcome. In a study in 1983, jack and lodgepole pine were successfully crossed in a nursery setting, to form a hybrid species (Kormutak, et.al., 1993). Jack pine x lodgepole pine crosses preformed by Dong et. al. (1992) indicate that crossing can be done successfully in either parental direction; jack pine as mother or father. Twenty-one matings between jack pine and lodgepole pine were conducted in the study, parents obtained from Alberta, British Columbia, and the Yukon Territory. One Alberta population sampled for parental DNA of jack pine used in Dong et. al. study (1992), was the Smoky Lake population used for obtaining jack pine samples in this study.

Natural hybridization is deterred between jack and lodgepole pine due to differences in flowering time; jack pine flowers 2-3 weeks before lodgepole pine. Ecological preference in

germinating site also varies between the two pines, making cross-fertilization difficult. Putative hybrids in Alberta have been observed to often produce aborted pollen grains or pollen grains that are smaller than non-hybrids. Forty-two percent of putative hybrids have aborted pollen as compared to only 1-2% in pure species, making hybrid proliferation difficult (Critchfield, 1985).

Although it is helpful to look at laboratory crosses to determine some hybrid characteristics, natural hybrid zones give a much better picture of the possibilities of genetic recombination. Laboratories are limited to only a few generations, where natural hybrid areas have been recombining genetic information for thousands of years (Barton and Hewitt, 1985).

1.1.7 Other Hybrids Occurring in Nature

There are many other examples of coniferous trees that hybridize naturally, so the hypothesis that lodgepole and jack pine may hybridize in the study area surrounding Fort Nelson, BC, is appropriate. Some good examples of other species that are suggested to naturally hybridize are Sitka x interior spruce (OECD, 2002) and black x red spruce (Johnsen, et.al., 1998).

Pinus sylvestris x *Pinus contorta* crosses occur in natural populations in Sweden, and are studied due to their commercial importance to Swedish forestry (Szmidt, et. al., n.d) for possible increases in population yield and tree vigor.

1.2 Thesis Objectives

This study was conducted in order to find evidence of hybridization between jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta* var. *latifolia*) in northeast British

Columbia, specifically the Fort Nelson region. Morphological evidence supporting hybridization in this area has been documented (Critchfield, 1985; Wheeler and Guries, 1987); however, substantial evidence of this interaction is yet to be shown. Using the principle morphological characteristics of hybrids according to literature, potential hybrid samples were collected for further investigation.

While attempting to characterize the data sets collected according to all of the objectives, it is essential that the characteristcs identified are related to one another in order to illustrate a picture of any hybrid samples that may exist within the data. Revealing how these trees are different from pure species, and what this means to the scientific and industrial communities as well as forest managers is of primary importance. Supportive evidence of hybridization and introgression in the Fort Nelson region of British Columbia will provide information for proper management of forests in this region, and specifications for processing and manufacturing. Objectives of this study were chosen to support morphological evidence of natural hybridization between jack pine and lodgepole pine.

The first objective of this study, and the focus of Chapter 2, was to genetically identify the paternity and maternity of each sample based on restriction fragment length polymorphisms (RFLPs). In this study, genetic evidence provides a basis for classifying species groups for further wood and fibre analysis, and wood extractive analysis.

The purpose of Chapter 3 was to present differences in morphology between species for proper identification of a species in the field, providing visual evidence of hybridization so noted in the literature, while categorizing species groups for comparison with genetic results from Chapter 2. Chapter 3 also quantifies variation in the morphology between species, reveals the relationship of some characteristics to site conditions, and determines the ability

of each characteristic to distinguish between species groups. This quantification provides a basis for comparison with other characteristics, namely wood and fibre traits (Chapter 4) and extractive chemistry (Chapter 5).

The objective of Chapter 4 was to compare species based on wood and fibre traits and to correlate these trait differences to genetic evidence and morphological differences between species and hybrids. Chapter 4 assists in determining if wood and fibre traits can be used as distinguishing features between jack pine, lodgepole pine, and their hybrids, while identifying interactions and relationships between wood traits, fibre traits and site conditions.

The primary goal of Chapter 5 was to determine if a "chemical extractive footprint" can be used to differentiate between jack pine, lodgepole pine and hybrid species. Characterizing the species in this way allows for further support of hybrid existence, and support the morphological evidence of hybridization.

1.2.1 Introduction to Species

Pure jack pine (*Pinus banksiana*) grows naturally from Great Bear Lake in Northwest Territories diagonally across Canada to southern Ontario, into southern Quebec, New Brunswick, and Nova Scotia (Farrar, 1995), while pure lodgepole pine (*Pinus contorta*) ranges over most of British Columbia and into Alberta. Lodgepole pine is found just over the Yukon border south through BC and well into the United States, and from the east side of the coastal mountains to western Alberta (Farrar, 1995), see Figure 2.

Both jack pine and lodgepole pine are hard pines that are self-pruning, meaning all branches are located at the top of the tree (Forintek Canada Corp., 1994). Pines are a primary successional species or pioneer species that grow best in deep, moist soils where drainage is good, but are usually out-competed by more shade tolerant species. Therefore these pine

species are often found occupying areas that other species are unable to inhabit such as rocky or very sandy soils (Isenberg, 1951). Lodgepole and jack pine usually inhabit open areas receiving plenty of direct sunlight and are susceptible to fire as a stand-replacing event every interval period depending on the location of the stand. Jack pine and lodgepole pine overlap in range in the northeast corner of BC, the southern Yukon and Northwest Territories, and in central Alberta (Forintek Canada Corp., 1994).

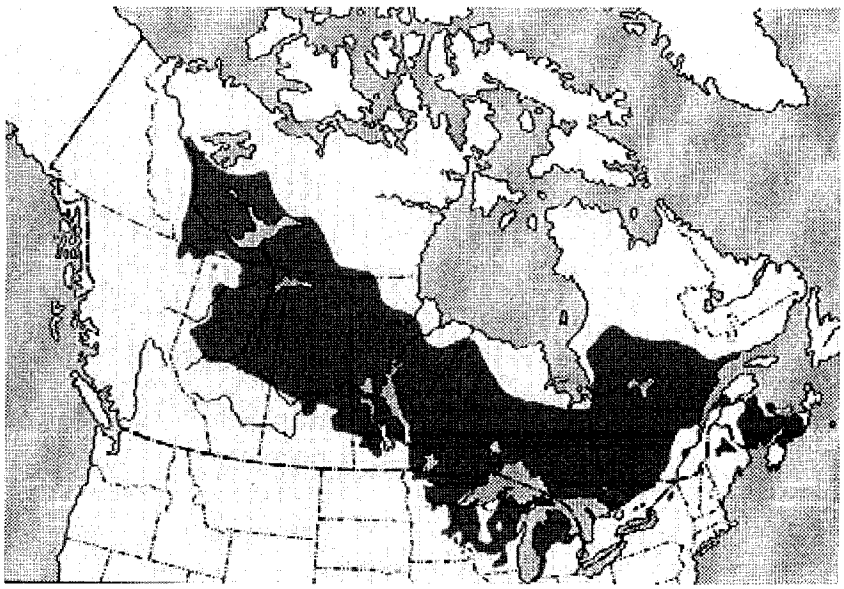

Figure 2a: Jack Pine Range (Farrar, 1995).

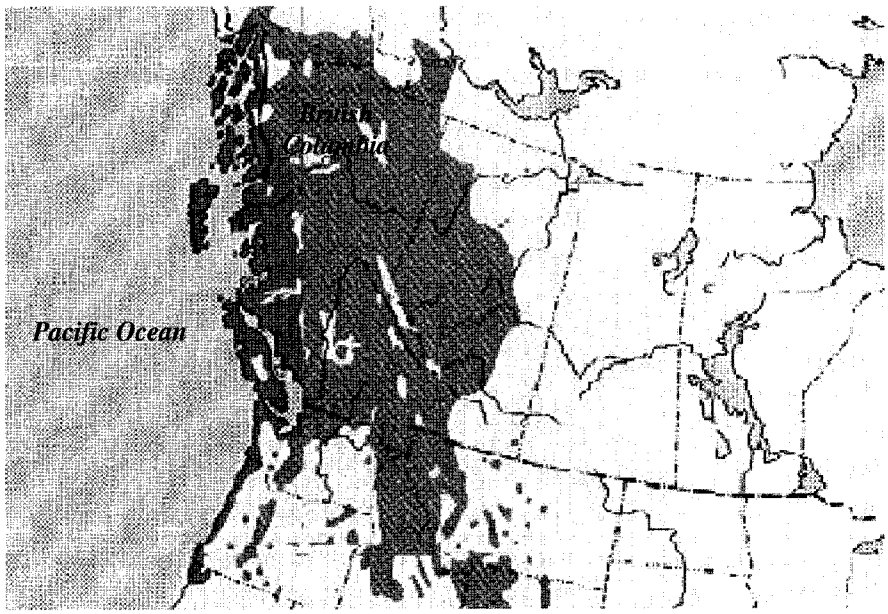

Figure 2a: Lodgepole Pine Range (Farrar, 1995).

1.3 Thesis Methodology

Pine samples were collected from three different areas. Jack pine samples were collected from central Alberta, east of the introgression zone; lodgepole pine samples were collected from the Prince George area of British Columbia, west of the introgression zone; and potential hybrid samples were collected from the Fort Nelson region of BC, within the introgression zone (further location and site descriptions can be found in Section 1.4). In order to classify each sample as lodgepole pine, jack pine, or hybrid, several characteristics were selected as potential identifying traits for each species group. These traits included gross morphological characteristics such as needle length, cone length, tree height, and tree diameter at breast height (DBH); wood and fibre morphology such as earlywood:latewood ratios, density, moisture content, cell size, modulus of elasticity, microfibril angle, fibre length, and fibre coarseness; and wood chemical extractive makeup.

To ensure that classification of species group was unbiased and to enable the evaluation of predictive abilities of certain species traits, the parental lines of each sample were genetically identified using restriction fragment length polymorphisms from chloroplast and

mitochondrial DNA. This allowed for genetic variation in species to be idenitified as well as possible hybridization.

Evaluation of each characteristic required various methods ranging from qualitative assessment to minute quantitative measurements. Height and DBH were measured and in the field, while all other measurements required sample collection for more detailed analysis, some using specific equipment. Characteristics requiring special preparation for measurement included wood and fibre traits, wood extractive chemistry, and genetic identity.

Wood and fibre traits were measured through scanning electron microscope, fibre quality analysis, and SilviScan analysis. Each of these methods required step by step sample preparation as outlined in detail in Chapter 4. The electron microscope was used to obtain images of the wood samples in order to distinguish visual differences between wood characteristics such as latewood content. Fibre Quality Analysis was preformed to obtain statistically correct measurements of fibre length and coarseness using wood material broken down into pulp form and then dispersed to a low concentration in water in order to measure individual fibres. SilviScan technology was used to obtain measurements for other wood characteristics that are more difficult to obtain, such as microfibril angle, modulus of elasticity, and average cell dimensions, using a combination of three different instruments, an x-ray densitometer, diffractometer, and image analysis equipment.

Wood extractive chemistry was investigated in this study through an in-depth process as described in Chapter 5. Using acetone, chemical constituents comprising the wood resin were extracted from sample wood cores and analyzed using gas chromatography – mass spectrometry. This process enables a spectral output to be obtained from each compound eluted. Each spectral graph displays the quantities of ions at various masses that were

present within each compound. This not only allows for possible identification of each compound based on its molecular weight, but also allows for quantification of each chemical within a sample. The chemical extractive make up of a particular sample can potentially be used to identify the wood species being tested. Some tree species produce unique quantities of certain compounds, which can then be used for identification purposes.

Chapter 2 outlines the protocol followed in order to obtain the genetic identities of each study sample. Chloroplast DNA from the *matk* gene (NCBI, 2004) was used to determine the paternity of each sample, and mitochondrial DNA from the *nad1* gene (Jaramillo-Correa et al., 2003) was used to determine the maternity of each sample. DNA was extracted from each sample, amplified using polymerase chain reaction, and digested using several restriction enzymes. Parental lines were determined by matching fragment length patterns of each sample from within the introgression zone, to those collected from outside the introgression zone (considered pure species).

By analyzing the variation between each sampling group with respect to each of these characteristics, the strength of the discriminatory ability of the characteristic was determined. To add to this, the ability of each characteristic to predict hybrid genotypes is discussed in further chapters.

The application of this study to forest management, breeding, and manufacturing and processing is discussed in Chapter 6.

1.4 Site Descriptions

1.4.1 Locations and Maps

This study involves three main forest stands and nine sites (three sample sites in each stand) within the species ranges mentioned above, seen in Figure 2. The first stand was located east of Smoky Lake, Alberta. This stand was a pure jack pine stand, even-aged at approximately 55 years old. This stand has been used by other researchers to obtain pure jack pine parent genotype and phenotype information (Dong, et. al., 1992; Danick and Yeh, 1983). The first of the three sites within the jack pine stand was located on Highway 28, east of Smoky Lake near the Smoky Lake Tree Nursery. Some dwarf mistletoe (*Arceuthobium americanum*) and western gall rust (*Endocronartium harknessii*) were present on trees in the stand but only endemically. The second jack pine sampling site was located across Highway 28 adjacent to an old burn area. Sampling site #3 was located on the eastern edge of the jack pine stand. Trees sampled were exposed on the southern face, while north sides of the trees faced into a densely arranged jack pine stand. Edge trees were more widely spaced, and possibly affected by wind and weather. Dwarf mistletoe was more prominent at this site than sites #1 and #2.

The second stand used for sampling was a lodgepole pine stand west of Prince George, BC. This stand was an even-aged mature lodgepole pine stand with an average tree age of 83 years. Three sites were chosen within this stand (sites #4, #5 and #6); two sites on the Bobtail Forest Service Road (FSR) (site #4 and 5), and one site on the Gregg Creek FSR (site 6). Site 4 was located 0.5 km south of Highway 16 on the Bobtail FSR. Site 5 within the lodgepole pine stand area was located at 11 km on the Bobtail FSR, north of Highway 16. The third site in the lodgepole pine stand (site 6) was located at 1 km on the Gregg Creek

FSR, south of highway 16. This site was adjacent to a previous cut-block with trees ages 8-10 years old. Otherwise this site was similar to sites 4 and 5. All three sites west of Prince George were subject to fairly heavy mountain pine beetle (*Dendroctonus ponderosae*) infestation. Trees were selected that did not demonstrate symptoms of attack. Site 6 was the most heavily infested of the 3 sites.

The third study area was located north of Fort Nelson, BC, bordering Alberta and the Yukon Territory. This area is distinct because it is the only area found in BC where the species range of jack pine and lodgepole pine overlap. Trees in this area ranged in age between 50 and 120 years old, with an average age of approximately 68 years. Sample sites for this study (site #7, 8, and 9) are located within this potential hybrid zone for jack pine and lodgepole pine. The first sampling site was located ~50 km north of Fort Nelson on FSR 7866-01 (the Patry Mainline) at 70.5 km mark. This site was primarily pine (~95%) with some spruce and a small deciduous component. The second sampling site was located five kilometers west of site #7 on the same FSR at 75.5 km mark, with the same characteristics. The third sampling site in the Fort Nelson region (site #9) was located approximately 40 km north of the Patry Mainline turnoff, on Highway 77. This site was a smaller even-aged pine stand composed of ~55% pine. Site maps are located in Appendix 1.

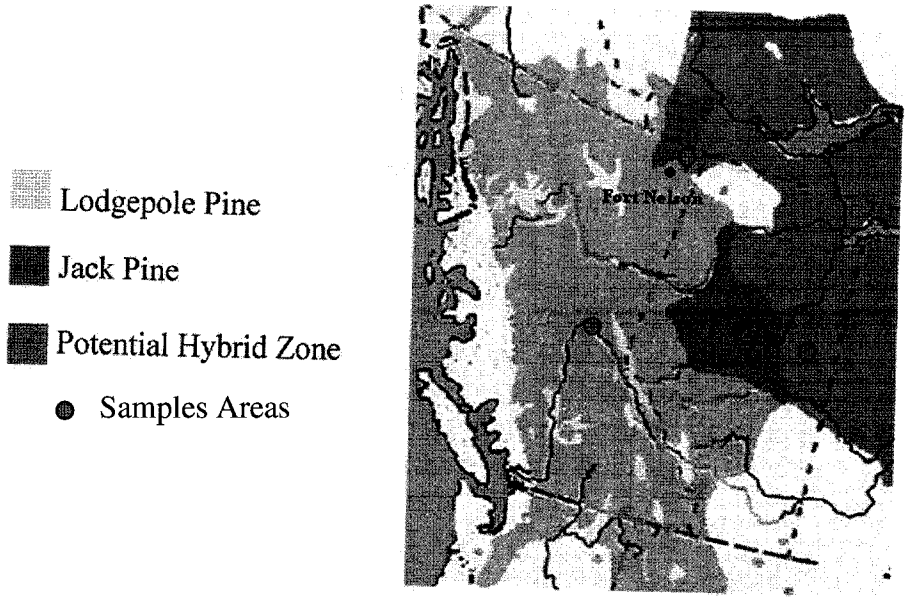

Figure 3: Sample area locations; 3 sites are located within each area
(from Sharma and Potter, 2000)

1.4.2 BEC Information

British Columbia is divided into 13 distinct biogeoclimatic (BEC) zones. Alberta does not have the same classification system, so the same descriptive facts do not exist for the sampling areas identified in AB.

- Smoky Lake sites selected for sampling are classified in the Bellis Lake and Bellis North Natural Areas which are described further in Section 1.4.3.
- Bobtail and Gregg creek FSR sites (west of Prince George) fall within the Sub-Boreal Spruce BEC zone, subzone moist-cool with a variant of 1(SBSmk1) (Meidinger and Pojar, 1991). Site series for the Prince George study sites is 03.
- The Fort Nelson sites selected for this study all fall within the BWBSmw2 biogeoclimatic zone, Boreal White and Black Spruce zone, moist and warm subzone with a variant of 2. It was determined using vegetation, soil descriptions, climate

information and topography of the area. Site series for the areas sampled is 02, representing the lodgepole pine forest cover of the area (Delong, et.al., 1990).

Site index was determined for the above sites in order to approximate the productivity levels of the stands being sampled. The site index value represents the average height of a dominant or co-dominant tree, in meters, growing on a particular site after 50 years (Stearns-Smith, 2001). Site index was determined to be 12.0 for the potential hybrid sites, 15.0 for the jack pine sites and 16.1 for the lodgepole pine sites, which suggests similar productivity.

1.4.3 Description of Soil and Vegetation

The first jack pine site in Smoky Lake, AB, has a very open understory with minimal vegetation composed of grey reindeer lichen (*Cladina rangiferina*), kinnikinnick (*Arctostaphylos uva-ursi*), and several types of moss. The second jack pine site had an understory composed of some deciduous and brush species including balsam poplar (*Populus balsamifera*) and alder (*Alnus spp.*). Vegetation on site #2 was composed almost entirely of grass species, with a small amount of grey reindeer lichen and moss. This site was slightly denser in stems per hectare than site #1 in the jack pine stand. The third site was quite densely populated with jack pine, and therefore little understory was present. Vegetation was almost entirely grass species.

All three jack pine sites were dry with well-drained, sandy soils. The first and third sites in the Smoky Lake area fell on the south side of Highway 28 and are therefore classified in the Bellis Lake Natural Area. The second study site for jack pine fell on the north side of the highway and was classified under the Bellis North Natural Area. Both natural areas contain soils of the Nestow soil series described as degraded Dystric Brunisols of loamy sand texture and aeolian origin, which are rapidly drained and have a fine layer of pine needles

overlying a variable Ae horizon. The soil profile for this area consists of loamy sand to sand with no discernable structure. The B horizon is brown, and lightens as it moves down to the C horizon. The pH of these soils is slightly acidic (Barnhardt, 2005).

The three sites west of Prince George were characterized as follows: Site 4 had an understory composed of some alder and trembling aspen (*Populus tremuloides*). Other brush species present included high bush cranberry (*Viburnum edule*), and red-osier dogwood (*Cornus stolonifera*). Vegetative species included fireweed (*Epilobium angustifolium*), and clasping twisted stalk (*Streptopus amplexifolius*). This was a mesic site with rolling terrain, slope varied between 0 and 10%. Soil drainage was moderate and of a finer texture than found in the Smoky Lake sites. Site #5 was very similar to site 4 only less dense. The understory vegetation was comprised of moss and grass species as well as bunchberry (*Cornus canadensis*) and Labrador tea (*Ledum groenlandicum*). Slope on this site was 0% throughout and drainage was moderate to poor. Soil had a greater clay content than site 4. Site 6, located at Gregg Creek, had a vegetative understory composed of clasping twisted stalk, grass species, and fireweed. Brush component included highbush cranberry and alder. Sampling site 6 was a mesic site with 0% slope throughout. Soils were moderately well-drained, and contained some clay content, similar to site 5. Nutrient regimes are classified as poor to very poor for the SBSmk1 site series 03 areas, and moisture regimes are subxeric (Delong et. al., 1993).

Potential hybrid sites #7, #8 and #9, located outside of Fort Nelson, BC, were characterized by a site series 02; dominant vegetation cover as primarily lingonberry (*Vaccininum vitis-idaea*) and velvet-leaved blueberry (*Vaccinium myrtilloides*). Tree cover was on average 40% composed of lodgepole pine, trembling aspen, black spruce (*Picea*

mariana), white spruce (*Picea glauca*) and jack pine. The shrub layer was representative at 30% cover, herb layer 50% cover, and moss layer 50% cover. Lodgepole pine was common on wetter sites in this area, in combination with black spruce, or in well-drained higher elevation sites. Moisture regime was classified as xeric to sub-xeric and nutrient regime is poor to very poor (Delong, et.al., 1990). Soils at study site 7 and 8 were classified as Kiwigana soils or eluviated dystric brunisols, associated with calcareous, silty, sandy, or gravelly fluvial materials. Soil texture was sand overlying very gravelly sand and drainage is rapid. Soils at study site 9 were classified as Sikanni soils or gleyed gray luvisols, associated with calcareous moderately fine-to fine-textured morainal materials. Soil texture was characteristic of silt loam overlaying clay loam. Drainage in this area was imperfect (Kowall, 1982).

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Chapter 2: Genetic Analysis of Jack Pine, Lodgepole Pine and their Hybrids

2.1 Chapter Objectives

The objective of this chapter was to genetically identify the paternity and maternity of each sample based on restriction fragment length polymorphisms (RFLPs). The identification of paternity and maternity of each sample provided recognition of first generation jack pine x lodgepole pine hybrids when results produced samples with divergent ancestry. After lineage identities were assigned to each sample the genetic information gathered was used to support the evidence of hybridization based on characteristics measured in the following three chapters.

Distinguishing hybrids from the pure species samples based on genetic analysis verifies the morphological evidence of hybridization in the Fort Nelson region and supports the presence of an introgression zone that has been previously documented in literature.

2.2 Support from Existing Literature

2.2.1 Description of CpDNA and MtDNA Markers

The markers used for determining hybridization between lodgepole pine and jack pine are polymorphisms located in the *mat*k chloroplast gene and in the mitochondrial gene, *nad*1. Polymorphisms are identified as a nucleotide difference at the same physical location on a genome. These differences allow inheritance of specific regions of the chromosome to be identified and traced as a genetic marker (Miesfeld, 1999). The chloroplast deoxyribonucleic acid (cpDNA) determines the paternity of the sample; inheritance of cpDNA is predominantly paternal in both lodgepole pine and jack pine. The mitochondrial deoxyribonucleic acid (mtDNA) determines the maternity of the sample; inheritance of mitochondrial genes is mainly maternal in these species (Dong et. al., 1992).

2.2.2 Critical Assessment of Various Markers

Many methods exist to analyze and compare hybridization between species. Random amplified polymorphic DNA (RAPD) markers, allozyme variability, the Eco2.0 probe, and chloroplast/mitochondrial DNA markers are all examples of ways to test for hybridization; these are discussed below.

RAPD markers are very abundant and accessible in most species, but limited in the amount of information derived from them due to their very short sequence length. These markers are however applied extensively to create genetic maps in many species (Yin, et.al., 2001).

Allozyme variation examined through electrophoresis can be used to identify evolution and variability in tree populations. Haploid tissue within the megagametophyte can be used for direct analysis and does not rely on controlled crosses (Danick and Yeh, 1983).

Eco2.0 is a probe developed to identify polymorphisms, and has been successfully applied to Sitka x Interior spruce hybrids (Potter, et.al., 2001). However, this probe has no assay developed for PCR amplification and therefore requires very high quality DNA from samples and analysis through Southern blotting, which has largely been replaced by polymerase chain reaction (PCR), and can be very time consuming.

By analyzing cpDNA and mtDNA through restriction fragment length polymorphisms (RFLPs) parental lines can be identified. CpDNA identifies father and mtDNA identifies mother, giving the ability to determine if the species in question is a hybrid or if it is pure. Because two types of DNA are required for this method, it can be more time consuming than using one region of DNA that can potentially give the same result, however, results are less prone to misinterpretation. In a study conducted on *Pinus contorta* and *Pinus sylvestris*

(Szmidt et. al., n.d.), it is suggested that little variation exists between individuals of the same population when looking at restriction patterns of cpDNA. However these areas are different when comparing between different species. This indicates that cpDNA restriction patterns are potentially useful for determining relationships in conifer phylogeny. Szmidt also supports that the chloroplast chromosome is ideal for determining phylogeny in plants because of its small size (only 150 kilobase pairs in total) and its absence of molecular heterogeneity. Perhaps the most convincing characteristic that allows the chloroplast genome to be a desirable area for phylogenetic study is its conservation over evolutionary time (Szmidt, et. al., n.d.)

2.3 Experimental Design and Hypotheses

Needles from foliar region of pines and inner cambial layers were harvested for DNA analysis. DNA was extracted from tissues and tested according to protocols listed in Section 2.4.

Thirty samples of pure lodgepole pine, 30 of pure jack pine, and 30 potential hybrid samples were collected from areas around Prince George, B.C., Smoky Lake, Alberta, and Fort Nelson region, B.C., respectively, yielding a total of 90 samples tested.

DNA sequences utilized throughout this procedure were obtained through GenBank Blast searches on the National Center for Biotechnology Information website (Oct.27, 2004), and from literature with the assistance of Dr. Craig Newton of Vizon SciTec Inc.

It is the belief of many forest scientists, and stated in numerous articles (Ye et. al, 2002; Kormutak et. al, 1993; Wagner et. al., 1992; Govindaraju et. al., 1988; Wheeler and Guries, 1987; Critchfield, 1985; Dancik and Yeh, 1983) that an introgression zone of jack pine and

lodgepole pine exists around the Alberta/British Columbia border, and that in this area, hybrids of these two species exist. Based on these previous assumptions, it is hypothesized that the parental lines of the samples collected within the introgression zone area will display that the maternity belongs to one species (either jack pine or lodgepole pine), and the paternity belongs to the other; thus indicating that the samples in the introgression zone are most likely F1 generation hybrids of the two pines.

More specifically, based on observations made at the time of sample collection in accordance with the morphology of the samples, it is believed that the samples collected from sites 7 and 8 in the Fort Nelson sampling area are more like jack pine; where as samples collected from site 9 in the Fort Nelson sampling area are more like lodgepole pine. It is questionable if this observation will be shown in genetic evidence or not.

The genetic analysis in this study was preliminary, and therefore, complex evolutionary details can not be identified from the results. If genetic recombination took place Fn generations ago, there may no longer be evidence of it at this analysis level. Therefore the percent make-up or contribution from each species to the potential hybrids will not be determined, the only question that will be answered is whether or not the parental lines of the samples are different species (indicating hybridization) or the same species (indicating pure species). This may or may not correlate to morphological evidence, as some characteristics will be strongly influenced by the environment, and some traits may not show up in hybrids after the F1 generation, due to back-crossing.

2.4 Methodology

2.4.1 Protocols for DNA Extraction

Using the protocol from the Qiagen DNeasy® Plant Mini Extraction Kit (Qiagen, 2004), needles and inner bark tissue were ground to a fine powder using liquid nitrogen and a mortar and pestle. Four hundred microlitres of Buffer AP1 were added to 1 ml of ground tissue, and mixed. The mixture was incubated for 10 minutes at 65°C and inverted 2 or 3 times during incubation. Following incubation, 130 µl of Buffer AP2 was added to the mixture, and the tubes were placed on ice for 5 min. The mixture was then centrifuged for 5 minutes at 14,000 rpm to separate lysates from precipitates. Lysate was then applied to the QIAshredder Mini Spin Column and centrifuged for another 2 min. at 14,000 rpm. Flow-through was transferred to a new tube without disturbing cell-debris pellets that may have formed during centrifuging. One and a half times volume of Buffer AP3/E was then added to the solution and mixed by pipetting. A maximum of 650 µl was then added to the DNeasy Mini Spin Column and was centrifuged at 8,000 rpm for 1 min. Flow through was discarded. This was then repeated until all of the solution had been run through the DNeasy Mini Spin Column. The column was then placed in a new 2 ml collection tube and 500 µl Buffer AW was added. The tubes were spun for 1 min. at 8,000 rpm. Flow-through was discarded and another 500 µl was added to the DNeasy Mini Spin Column and spun down for 2 min. at 14000 rpm to dry the membrane. The DNeasy Mini Spin Column was then transferred to a new tube and 100 µl of Buffer AE was pipetted directly onto the membrane. Tubes were incubated for 5 min. at room temperature and centrifuged for 1 min. at 8,000 rpm to elute. The elution was repeated to yield a DNA dilution in 200 µl of Buffer AE.

Using the phenol – chloroform protocol for DNA extraction according to Newton (2004), needle and inner bark tissue was ground to a fine powder under liquid nitrogen, adding polyvinyl-polypyrrolidone as needed. One thousand micro-liters of extraction buffer were added to the ground material and vortexed to mix. The mixture was incubated at 65 °C for 15 min. Seven hundred and twenty micro-liters of chloroform isoamyl alcohol were then added and the mixture was centrifuged at 14,000 rpm for 5 min. Aqueous layers were carefully removed from the mixture and transferred to a new 1.5 µl tube. Approximately 10% volume of ammonium acetate was then added and the solution was mixed by inversion. Isopropanol was added to the mixture at 1.1 x volume and the solution was again mixed by inversion. The tubes were spun down at 7,500 rpm for 5 min. Isopropanol was removed from the tube being careful not to disturb the pellet formed at the bottom of the tube. The pellet was washed with 70% EtOH, tubes were spun down, EtOH was removed, and tubes were left to air dry. Product left in the tube was then dissolved in 100-200 µl TE buffer.

2.4.2 DNA Quality and Quantity

All DNA samples were assessed for the quality of DNA present in the sample and for the quantity of DNA available within the sample. Quality of DNA was assessed through electrophoresis, quantity was assessed through testing of various dilutions of DNA in PCR amplifications.

2.4.3 Polymerase Chain Reaction Analysis

Polymerase chain reaction (PCR) amplification was used to copy select sequences so larger quantities of DNA could be used for further processes. Lodgepole pine and jack pine sequences were compared, and primer pairs and restriction enzymes chosen to be species

specific markers. In order to identify the portion of DNA needed for amplification, several primers were used. The cpDNA was processed using two different primer pairs obtained through GenBank Blast searches; one primer pair to amplify cpDNA Section 1, from nucleotide 1 to 540 on the *mat*k gene (See Appendix 2), and one to amplify cpDNA Section 2 from nucleotide 1081 to 1660 on the *mat*k gene (See Appendix 2). Restriction enzymes cut jack pine cpDNA while leaving lodgepole pine cpDNA in one full sequence. The mtDNA was processed using three different primer pairs obtained from literature. The first was used to amplify a portion of the *nad*1 gene, the second was to amplify a section of the *nad*4 gene, and the third was to amplify a portion of the *nad*5 gene. An attempt was made to optimize each PCR in order to gain the best result possible and yield the most DNA for digestion. Only *nad*1 gene material was successful during amplification and used for obtaining digested fragment patterns which were species specific.

The protocol established by Newton (2004) for PCR was used for the cpDNA reactions. Protocols from literature (Jaramillo-Correa et. al., 2003; Kubo et. al., 2000; Demesure et. al., 1995) were used initially for the *nad*1, *nad*4, and *nad*5 regions, with minimal to no results, therefore requiring optimization. Optimization of the $MgCl_2$ concentration was preformed first. Trials of concentrations ranging from 1.6 mM to 2.4 mM of $MgCl_2$ in a 12.5 µl reaction were preformed. Following this, the optimal $MgCl_2$ concentration was utilized while annealing temperatures were varied for optimization. A PCR trial was set up with samples subjected to annealing temperatures that varied on a gradient from 42°C to 55°C. The annealing temperature that produced the best product was utilized for the full reaction of all samples in *nad*1 using the PCR protocol by Newton (2004),

however, no results were obtained for the *nad*4 and *nad*5 reactions even after optimization, so these gene sequences were not used.

CpDNA primers for PCR amplifications are as follows:

- PbMatSnaBF – 5' ATGGATGAGTTCCATAGATG 3'
- PbMatSnabR – 5' AACAGATCGTAATGGGTGCA 3'
- PbMatHhaF – 5' ATTCTGTGACATATCAGGGC 3'
- PbMatHhaR – 5' TTCTCATTGCACACGGCTTT 3'

MtDNA primers for PCR amplifications are as follows:

- nad5aF – 5' AGTCCAATAGGGACAGCAC 3'
- nad5aR – 5' ACCCGACGATAACTAGCTTC 3' (Jaramillo-Correa et. al., 2003)
- nad4L-orf25F – 5' TATTACTTTCCGAGTCCGGGG 3'
- nad4L-orf25R – 5' TCTTCTTCGAACTTGATGCAC 3' (Kubo, et. al., 2000)
- nad1exonBF – 5' GCATTACGATCTGCAGCTCA 3'
- nad1exonCR – 5' GGAGCTCGATTAGTTTCTGC 3' (Demesure et. al., 1995)

A. Primer Preparation:

DNA/RNA free H_2O was added to the primer tubes at ten times the initial primer volume to resuspend the oligonucleotide. Five µl of 50nM forward primer and 5 µl of 50nM reverse primer were mixed together and 90 µl of H_2O was added to further dilute the primer mixture to 0.5 nM.

B. DNA Preparation:

DNA was further diluted to 1/100 µl in DNA/RNA free H_2O. This diluted DNA concentration was used for chloroplast PCR amplifications and the initial *nad*1 PCR

amplification. Secondary *nad*1 PCR amplifications were preformed using DNA concentrations in their original TE dilutions, with no further dilution due to some DNA degradation over the time frame in which the DNA was used.

C. Amplification:

For 12.5 µl PCR reactions, 1.25 µl of 10x buffer was mixed with 0.75 µl 25 mM $MgCl_2$ (varying depending on optimized concentration), 0.2 µl dNTPs (20 mM each), 1.0 µl DNA at varying concentrations depending on optimal, 1.0 µl primer (F/R mix at 0.5nM), 0.3 µl platinum *taq* polymerase (5 units/µl), and 7.5 µl of H_2O (or amount to top to 12.5 µl). Some reactions were made at 50 µl (same proportions as above) in order to create enough product for digestion by restriction enzymes. Master mix of buffer, $MgCl_2$, dNTPs, *taq*, and water was made based on number of samples prepared. DNA and primer were added separately to each tube. CpDNA primers were used to successfully amplify product using the following procedure: 95°C for 3 minutes, 95°C for 30 seconds, 55°C for 15 seconds, 72°C for 1 minute, repeat steps 2-4 for 35 cycles, 72°C for 5 minutes (Newton, 2004). MtDNA *nad*1 primer product was successfully amplified using the following optimized PCR procedure: 95°C for 3 minutes, 95°C for 30 seconds, 55°C for 15 seconds, 72°C for 1 minute, repeat steps 2-4 for 35 cycles, 72°C for 5 minutes. MtDNA *nad*4 and *nad*5 primer products were unsuccessful in amplification after several attempts using procedures from literature, variations in these procedures regarding annealing temperature and extension time, and variations in $MgCl_2$ concentration and DNA concentration. Procedures according to Newton (2004) were also unsuccessful for *nad*4 and *nad*5. Negative control samples were run to ensure mixtures were not contaminated.

2.4.4 Restriction Digest

For the cpDNA *mat*k gene, restriction enzymes *SnaB*1 and *Hha*1 were used to cut the DNA at TACGTA and GCGC, respectively (See Appendix 2). Digestion of the mtDNA *nad*1 fragment was necessary in order to interpret the difference between the species after PCR amplification of the large fragment. This digestion was preformed using three restriction enzymes: *Rsa*1, *Mbo*1, and *Hha*1.

Jack pine cpDNA displayed 2 DNA fragments at different base pair weights when cut with *SnaB*1 and *Hha*1, while lodgepole pine cpDNA remained in one fragment. The variation in number of fragments after digestion identified the paternity of the samples.

The *nad*1 gene of mtDNA was digested using *Rsa*1, *Mbo*1, and *Hha*1. Each formed a unique banding pattern which identified variation between species. The variation in banding pattern after digestion identified the maternity of the samples. Banding patterns formed from the three digests were analyzed by comparing bands with those that existed in the original amplified segment of DNA. Smaller sized bands that were conserved from the original DNA segment and appear in all the digested material were eliminated from the analysis. These bands are most likely caused by non-specific primer binding with other sites in genome that results in amplified by-product, and was not the targeted material.

In order to ensure that digests were complete, all true product band lengths were added to equal the length of the original PCR product. This length for the cpDNA products was 540 base pairs (bp) for cpDNA Section 1, and 580 bp for cpDNA Section 2; mtDNA product was approximately 2000 bp.

After determining the maternity and paternity of each sample based on the restriction fragment length polymorphisms it was possible to piece together the parental line of each sample and identify if any hybrids existed within the sample sets.

2.4.5 Imaging

After running DNA samples on agarose gels stained with EtBr, the electrophoresis output was determined using an ultra violet image analysis tool. This allowed digital images of the gels to be viewed and saved electronically.

2.5 Results

The following images display the gel electrophoresis output from DNA extraction, amplification, and digestion. Illuminated bands indicate a presence of DNA at a certain weight in base pairs.

Table 2: Sample numbers as collected from each sampling area.

Sample #	Species Area
1 - 30	Jack Pine
31 - 60	Lodgepole Pine
61 - 90	Potential Hybrid

2.5.1 DNA Extraction

Samples 1 through 23 and 27 were initially extracted using the chloroform/phenol method outlined in Section 2.4.1. Bands were faint using this extraction procedure indicating less than optimal extraction amounts, therefore a Qiagen® plant mini DNA extraction kit was used for the remainder of the samples, and only samples with acceptable output were kept from the chloroform/phenol method. All samples extracted using Qiagen® plant mini DNA extraction kit yielded good DNA output. Figure 4 displays DNA obtained from this

extraction along with labeling for sample numbers. Figures thereafter are not labeled, but sample numbers are given in the caption and numbering follows the same method, left to right and top to bottom.

The only samples without visible product from extraction were 33, 36, 37, 42, 57, 58, and 60. Sample 11 was lost and therefore none of the results include sample 11. All samples were used for PCR regardless of product visibility in initial extraction output.

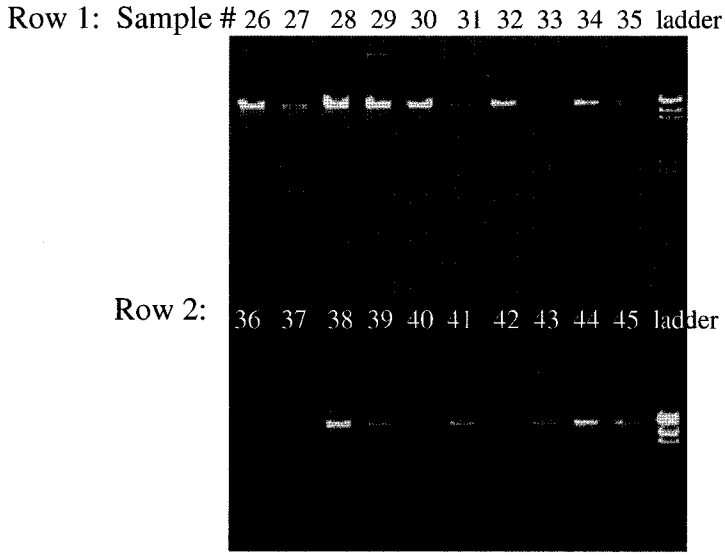

Figure 4: Extraction of DNA, row 1 left to right; samples 26-35, λ Hind III ladder, and row 2 left to right; 36-45, λ Hind III ladder.

2.5.2 PCR Products

DNA extractions were diluted to $1/10^{th}$ and $1/100^{th}$ the concentration and amplified as outline in Section 2.4.2. Most samples were amplified successfully. Some DNA samples degraded over time and were therefore not amplified and excluded from the digestions, these samples are: 1, 3, 4, 6, 7, 10, 12, 17, 33, 34, 57, 63, 64, 74, 76, 82, and 84. For subsequent statistical analysis, it was assumed, due to similarity in sampling location and therefore pollen and seed sources, that these samples follow similar fragment patterns to those digested

and displayed in Section 2.5.3. Some amplifications revealed faint, non-specific banding at lower fragment base pair weights. However this does not interfere with the product distinction and were taken into account in the analysis of restriction fragment patterns.

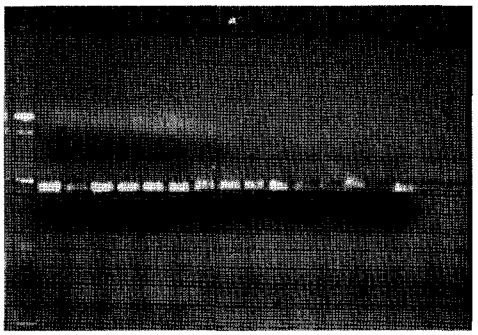

Figure 5: CpDNA Section 1 PCR product (refer to Appendix 2). Wells left to right; 100 bp ladder, samples 72-86.

Figure 5 displays the PCR product for cpDNA Section 1, which was subsequently digested with the *SnaB*1 enzyme. In most samples, the amplified fragment of DNA showed up well on the gel output. The desired fragment was 540 base pairs in size, as expected.

Row 1:

Row 2:

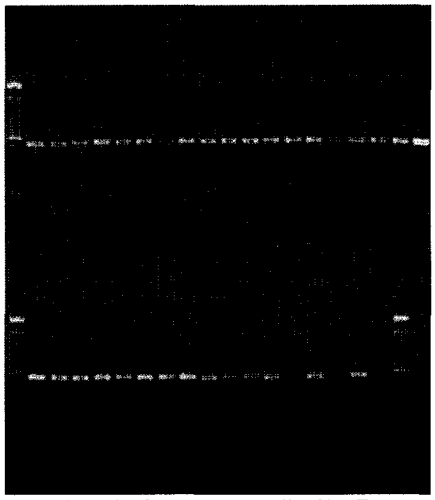

Figure 6: CpDNA Section 2 PCR product (refer to Appendix 2). Row 1, wells left to right; 100bp ladder, sample 2-21 (no 11). Row 2, wells left to right; 100 bp ladder, samples 22-38 (no 31), negative control, 100 bp ladder.

Figure 6 shows an example of the PCR product obtained for cpDNA Section 2, which was subsequently digested with the *Hha1* restriction enzyme. Most of the samples amplified well, and produced a clear fragment band 580 base pairs in length.

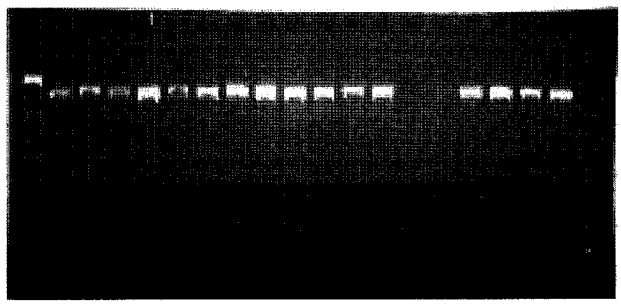

Figure 7: *Nad*1 PCR product (optimized annealing temperature of 55°C). Wells left to right; 1 kb ladder, sample 13, 14, 43, 44, 73, 74, 15, 16, 45, 46, 75, 76, 17, 18, 47, 48, 77, 78.

Figure 7 shows the *nad*1 PCR product with the optimized DNA concentration and annealing temperatures. Most samples produced a clear band around 2000 base pairs in size. The bands show no distinguishable size difference between species groups. This trial was preformed at PCR conditions as per Newton (see section 2.4.2), with a 55°C annealing temperature.

2.5.3 Restriction Digests

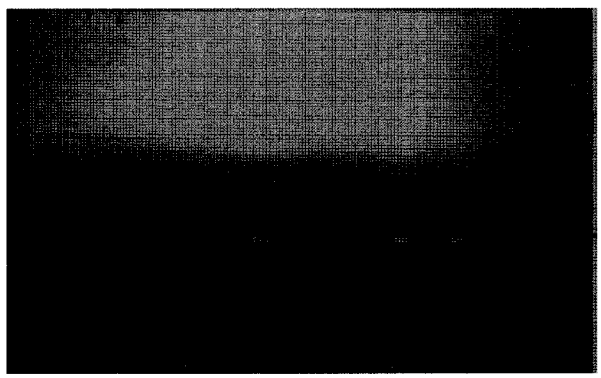

Figure 8: CpDNA Section 1 PCR product digested with *SnaB*1 enzyme. Wells left to right; sample 59-77, 1 kb ladder.

Figure 8 shows the digestion of Section 1 of cpDNA *mat*k gene PCR product by the restriction enzyme *SnaB*1. Samples 1 through 30, and 61 through 80 were digested into 2 small size fragments the first weighing approximately 320 base pairs, and the second weighing approximately 220 bp, as shown in Figure 8. Samples 31 through 60, and 81 through 90 were not digested and remain in one product band. The undigested bands are of the same size as the original PCR product (540 bp). Some bending in the wells occurred during electrophoresis, which accounts for the uneven placement of the bands. This is a source of experimental error and was probably due to impure TBE buffer in which the electrophoresis was preformed.

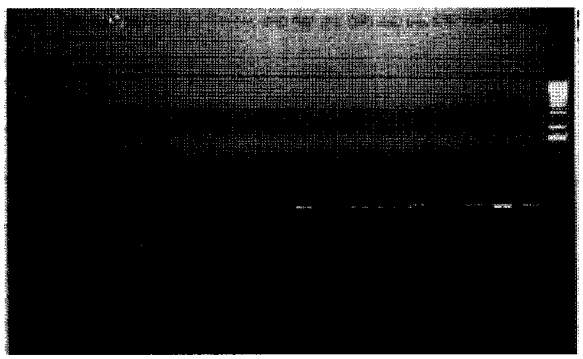

Figure 9: CpDNA Section 2 PCR product digested with *Hha*1 enzyme. Wells left to right; sample 21-39, 1 kb ladder.

Figure 10: CpDNA Section 2 PCR product digested with *Hha*1 enzyme. Wells left to right; 1 kb ladder, sample 78-90.

Digestion of Section 2 of the *mat*k gene with the *Hha1* enzyme supports the Section 1 digestion results, showing the same digestion pattern of samples (Figure 9 and 10). Samples digested compared to those undigested are clearly seen in these figures. Samples 1 through 30 and 61 through 80 were digested into 2 small size fragments, 300 bp and 280 bp; samples 31 through 60 and 81 through 90 were not digested. The large band remaining in the undigested samples is the same size as the band in the original PCR product (580 bp). The results from this digestion coordinate exactly with the results from the *SnaB*1 digestion, shown in Figure 8.

As expected, the jack pine samples were all digested in both the cpDNA reactions. None of the lodgepole pine samples were digested in these reactions. This variation in digestion provides a basis for comparison of putative hybrid samples. The potential hybrid pine samples showed varying results. Samples 61 through 80, from the first 2 sites in the Fort Nelson area, show paternity of jack pine (digested bands); samples 81 through 90, from the third site in the Fort Nelson area, show paternity of lodgepole pine (undigested bands). Each sample was assigned a haplotype in order to compare banding patterns for proper identification of paternity as well as to identify any unique samples, refer to Table 3.

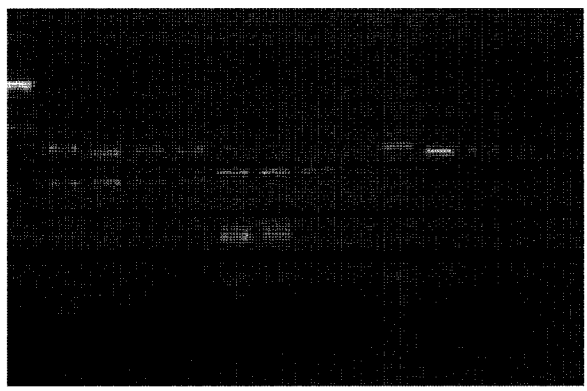

Figure 11: *Nad*1 PCR product trial digestion using *Rsa*1, *Mbo*1, and *Hha*1. Wells left to right; 1kb ladder, sample 20 H, 46 H, 73 H, 81 H, 20 R, 46 R, 73 R, 81 R, 20 M, 46 M, 73 M, 81 M.
H = *Hha*1, R = *Rsa*1, M = *Mbo*1

Figure 11 shows the digestion of the *nad*1 gene PCR product by *Rsa*1, *Mbo*1, and *Hha*1 restriction enzymes. Each enzyme cut the PCR product into several fragments, forming different banding patterns depending on the enzyme. Based on this trial, sample 20 (jack pine) displays a different banding pattern then samples 46 (lodgepole pine), 73 (potential hybrid), and 81 (potential hybrid). This indicates that the potential hybrid samples match the lodgepole pine banding pattern for the mitochondrial gene (*nad*1).

Some sample digestions have additional bands which can not be explained without further analysis. For example, sample 59 contains a fragment at approximately 400 bp which is found in every digest (*Rsa*1, *Mbo*1, and *Hha*1). This fragment is not found in the original PCR product, but could be mtDNA material which was not intended to be amplified. It is not considered to be a digested product fragment, forming an alternate haplotype, because this band was consistantly found throughout each digestion, and the addition of this fragment would bring the total digested fragment weight to well over that of the original amplified product (~2000 bp). Other bands were incompletely digested, also evident because of expected total band weight for each sample. These "suspicious" bands were not considered in the DNA analysis.

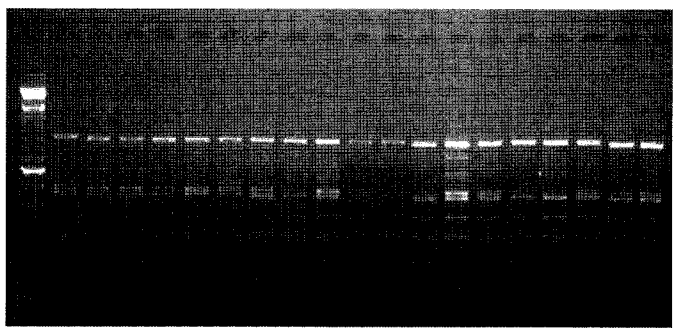

Figure 12: *Nad*1 PCR product digestion using *Rsa*1 (left to right): 100 bp ladder, sample 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44.

The digestion using restriction enzyme *Rsa*1 reveals that different haplotypes exists for each species group; haplotypes are displayed in Table 4. There are distinctive bands present to distinguish between species, and the absence of a bright band at approximately 360 base pairs distinguishes most jack pine samples (samples #1-30) from the rest of the samples. However, the 360 bp band is present in 4 of the jack pine samples (sample 19, 27, 28, and 29). All the potential hybrid samples (61-90) have banding patterns most closely matching the lodgepole pine samples (31-60).

The band present at ~500 bp in almost all samples was disregarded due to its presence in the original PCR amplified product, indicating that it is not a product of the intended digest.

The presence of an extra band in the lodgepole pine and hybrid samples (as well as the four unique jack pine samples) makes the total weight of the digested fragments to ~2250 base pairs, which is larger than the total weight of the digested jack pine fragments (~1900 bp). This difference could be accounted for by smaller fragments in the jack pine samples which are not visible due to the expected fainter staining of smaller sized bands and/or the migrations of the EtBr stain which runs in opposite direction to the samples, and therefore does not highlight lower regions of the gel where smaller bands would be located.

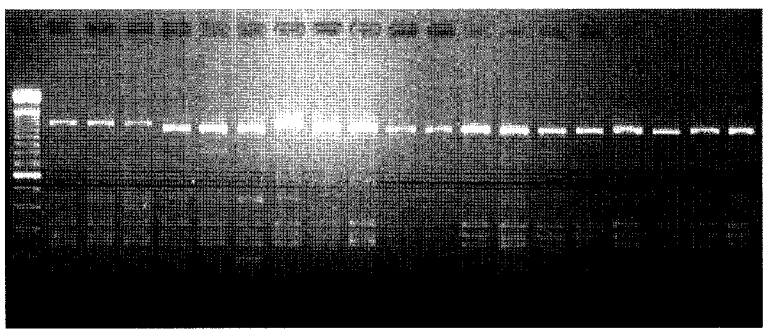

Figure 13: *Nad*1 PCR product digestion using *Mbo*1 (left to right): 100 bp ladder, sample 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44.

Most jack pine samples have a distinctive band at approximately 1200 base pairs, where as all lodgepole pine samples and the potential hybrid pine samples have a band at approximately 1100 base pairs. Jack pine samples 19, 27, 28, and 29 have the same band pattern as the lodgepole pine samples, as previously seen in the *Rsa*1 digestion (Figure 12).

In this digest the jack pine samples have a larger fragment present in the digest than lodgepole pine samples (1200 bp and 1100 bp, respectively). This may indicate that the original PCR product for jack pine was slightly larger than lodgepole pine, however was not distinguishable based on the size of the fragment and combined electrophoretic output. It is also possible that smaller bands, whose presence in lodgepole pine samples would make the total weight of the digested bands equivalent to that of jack pine, are not distinguishable in the digests. This is also true for the digest using *Hha*1, as seen in Figures 14 and 15.

As stated in the digest for *Rsa*1, the band present at ~500 bp in almost all samples was disregarded due to its presence in the original PCR amplified product, indicating that it is not a product of the intended digest.

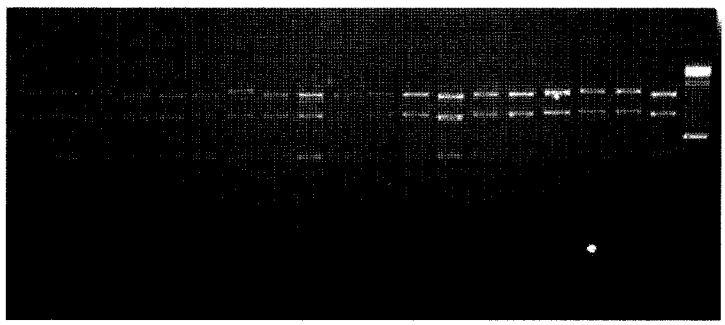

Figure 14: *Nad*1 PCR product digestion using *Hha*1 (left to right): sample 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 100 bp ladder.

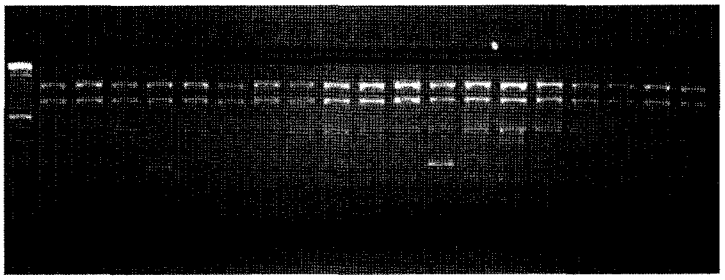

Figure 15: *Nad*1 PCR product digestion using *Hha*1 (left to right): 100 bp ladder, sample 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 65, 66.

Digestion with the *Hha*1 restriction enzyme revealed species specific banding patterns. Jack pine samples have a distinctive band at 1350 base pairs, and lodgepole pine samples have a band at approximately 1250 base pairs.

Potential hybrid samples have the same band as the lodgepole pine samples, paralleling the results of the previous two restriction enzymes (*Rsa*1 and *Mbo*1). The same four jack pine samples display lodgepole pine banding as seen in *Rsa*1 and *Mbo*1 digestions. As stated for the other digests, the band present at ~500 bp in almost all samples was disregarded due to its presence in the original PCR amplified product, indicating that it is not a product of the intended digest.

Jack pine banding patterns can be differentiated from lodgepole pine banding patterns according to the results from the mtDNA *nad*1 gene digestions. All of the potential hybrid samples most closely match the lodgepole pine bands indicating that the maternity of the potential hybrids is lodgepole pine. It is also evident that the maternity banding pattern of jack pine samples 19, 27, 28, and 29 is unique, with bands that are found in both pure species.

All *nad*1 digestions were classified according to haplotype, shown in Table 4. Each haplotype identifies a different banding pattern. Based on these groupings, parental identity and similarities or differences between samples can be easily distinguished.

Haplotypes:

Table 3: Paternity haplotypes for each species group broken down by collection site. Number indicated in the "sites" column represents the number of the corresponding haplotype that was found within the samples from that site. For example, Hybrid site 8 has 7 samples with the haplotype AA.

			Jack pine sites			Hybrid pine sites			Lodgepole pine sites		
Haplotype Number	***SnaB*1**	***Hha*1**	**1**	**2**	**3**	**7**	**8**	**9**	**4**	**5**	**6**
cp1	A	A	4	4	6	8	8				
cp2	B	A		3							
cp3	C	A			4						
cp4	D	B						8	8	10	10

Table 4: Maternity haplotypes for each species group broken down by collection site. Number indicated in the "sites" column represents the number of the corresponding haplotype that was found within the samples from that site. For example, Jack pine site 1 has 4 samples with the haplotype AAA.

				Jack pine sites			Hybrid pine sites			Lodgepole pine sites		
Haplotype Number	***Rsa*1**	***Mbo*1**	***Hha*1**	**1**	**2**	**3**	**7**	**8**	**9**	**4**	**5**	**6**
mt1	A	A	A	4	6	7						
mt2	B	B	B		1	3	8	8	8	8	10	10

Tables 3 and 4 reveal similarities between maternity and paternity within some samples. There are also a few samples with distinct haplotypes. Hybrid samples from site 9 have both maternity and paternity matching lodgepole pine samples, which has unique implications. Jack pine samples with an mt2 haplotype are unique; these findings will be discussed in Section 2.6. In total 16 out of 24 hybrid samples had a cp1 paternal haplotype and an mt2 maternal haplotype, matching jack pine paternity and lodgepole pine maternity.

2.6 Discussion and Conclusions

It was found in the cpDNA digestions that samples 1-30 possess a confirmed paternity of jack pine and samples 31-60 posses a confirmed paternity of lodgepole pine. These samples also have a maternity matching their respective species with the exception of sample 19, 27, 28, and 29, which were expected to have a maternity of jack pine but in fact seem to have banding patterns that indicate a lodgepole pine maternity. These samples (19, 27, 28, and 29) have genetic patterns of possible hybrids, and for statistical analysis of traits they will be classified as species group 4. Due to the fact that samples 19, 27, 28, and 29 all possess a paternity of jack pine and banding patterns with lodgepole pine maternity (mt2 haplotype), it can be assumed that these specific samples may also be natural hybrids, perhaps from an introgression event that has been developing over centuries. It is possible that these trees underwent hybridization many generations ago, and since then have been back-crossed with pure jack pine trees, losing the distinct F1 polymorphism that is used to distinguish hybrids through restriction digestion in this study; although these trees have all morphological traits resembling only jack pine which distinguishes them from other hybrids studied. This may also be an indication that hybrids and introgression took place further east in Alberta than documented.

Samples 61-80 (excluding samples 63, 64, 74, and 76) have paternity matching the jack pine samples and maternity matching the lodgepole pine samples indicating that these species are most likely a natural hybridization of the two pine species. Excluded samples are expected to follow the same trend in analysis and were excluded because of degradation of DNA during laboratory processes. These hybrids can be interpreted as F1 generation hybrids because they show direct lineage to pure species groups. However, these trees would also

display a jack pine paternity and lodgepole pine maternity in 50% of the F2 genotypes, and 50% of backcrossed genotypes.

Samples 81-90 (excluding samples 82 and 84) have a paternity matching lodgepole pine samples and a maternity also matching lodgepole pine, indicating that these samples, although morphological resembling natural hybrids for some characteristics such as cone angle and curvature, do not have the genetic banding patterns to support hybridization in the F1 generation. Excluded samples are expected to follow the same trend in analysis and were excluded because of degradation of DNA during laboratory processes. More genetic analysis would have to be conducted on samples 81-90 in order to determine if any hybridization occurred to form the physical characteristics that these samples possess. It is possible that these trees are hybrids but do not represent the F1 generation, and therefore do not possess the distinct polymorphism that was used for restriction digestion in this study. These samples may have been produced from hybrids that were back-crossed with lodgepole pine trees in the surrounding area, and therefore genetic evidence of hybridization is indistinguishable at this level of analysis. For statistical analysis of traits, samples 81-90 will be classified as species group 5.

The results for samples 19, 27-29, and 81-90 leave room for interpretation, as the banding patterns visible for these samples are unique and could represent within species variation or a possible past introgression event; a more in depth genetic analysis is required in order to further explain these specific sample cases (Refer to Appendix 2). Evidence that indicates that these four samples are different from other jack pine samples is clear in Tables 3 and 4. Samples 19, and 27-29 have cpDNA haplotypes matching other jack pine samples (cp1), but mtDNA haplotypes matching lodgepole pine samples (mt2). Although these

samples seem like F1 generation hybrids, they are morphologically identical to other pure jack pine samples, and therefore suggest a past introgression with pure jack pine. Evidence of introgression in this area indicates that the Alberta introgression zone may extend further East than previously thought. Samples 81-90 are also good examples of possible past hyrbidization with back-crossing was lost likely with lodgepole pine based on banding patterns and trees found in the immediate surrounding area.

Slight within species variation was expected and shown in results as variation in haplotype; cp1, 2 and 3 show variation in jack pine samples (Table 3). Variation in haplotype could be due to individual tree differences caused by adaptation to environmental gradients, or due to possible introgression of genes over generations. Back crossing in populations of both species could potentially disguise genetic inheritance. Hybrids could be present that do not show up from digestion because of too much introgression, and dominance by one species over the other. For these hybrids to be identified, further genetic investigation is required. It is not surprising that some samples were found with morphologic characteristics of hybrids but a pure species genotype. Introgression and creation of clines (as discussed in Section 1.3.1) means that a genetic gradient exists within the hybrid area (Woodruff, 1973). For this study, the cline can be most closely characterized as a parapatric zone because while jack pine populations remain localized from lodgepole pine populations; hybrid stands form between parental pure species stands, and are somewhat integrated (refer to Figure 3 and Table 1). According to Barton and Hewitt (1985), a parapatric population indicates that full equilibrium between pure species populations and hybrid has not been reached, which is supported by the existence of F1 generation hybrids in the Fort Nelson region, that must have been crossed within the last 150 years.

The genetic evidence in this study supports the presence of hybrid jack pine x lodgepole pines. It can be concluded based on the genetic evidence, that there is hybridization between lodgepole pine and jack pine occurring in the Fort Nelson region of British Columbia. Assumptions can be made that species crosses exist where morphological evidence occurs, and that tree displaying morphology of both species could eventually be identified as genetic hybrids with the appropriate laboratory analysis.

In following chapters, morphology, wood chemistry, and wood and fibre properties are discussed. Samples from each sampling area were used for comparison of various tree characteristics. For this comparison, it is useful to look at the variation among species groups as distinguished by area: 1) Smoky Lake – jack pine 1-30, 2) Prince George – lodgepole pine 31-60, 3) Fort Nelson – hybrid pine 61-90; and also as distinguished by genetic RFLP group: 1) jack pine 1-18, 20-26, 30, 2) lodgepole pine 31-60, 3) hybrid pine 61-80, 4) unique jack pine haplotypes 19, 27-29, and 5) possible Fn generation hybrids 81-90.

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Chapter 3: Morphological Analysis of Jack Pine and Lodgepole Pine

3.1 Chapter Objectives

The main purpose of documenting differences in morphology between species is for proper identification of a species in the field and also because of its traditional importance in biological study. It is important to distinguish between lodgepole pine and jack pine in order to recognize the presence of a hybrid lodgepole pine x jack pine tree. Literature available that described hybrid characteristics in AB and NWT was used in order to identify areas where hybridization between jack pine and lodgepole pine was plausible in Northern B.C. Based on the literature, morphological characteristics that would most easily distinguish possible hybrid individuals were identified, such as crown shape and cone orientation. Since tree samples in the introgression zone around Fort Nelson were selected for their morphological traits, resembling possible hybrids, it is necessary to measure how strong the hybridization actually is by comparing these possible hybrid samples with samples collected based on "pure species" characteristics. It is possible that some level of intermediacy will be identified within the hybrid characteristics, indicating the presence of both jack pine and lodgepole pine influence.

Morphology of pure species and hybrids are investigated to measure ability to distinguish between pure species and hybrids, and provide a basis for comparison with other characteristics, namely wood and fibre traits, extractive chemistry, and genetic variability.

3.2 Species Characteristics According to Existing Literature

Conifers can be classified by several distinguishing features. Morphological characteristics that are most commonly used to identify species of conifers, specifically pines, include: cones, needles, bark, and height and crown shape.

Lodgepole pine is characterized by cones oriented perpendicularly from the stem (Farrar, 1995). Some cones are serotinous; cone scales are fixed together with resin, so seeds are sealed until released via a heat interaction of 45-50^{O} C, refer to Figure 17 (Koch, 1996).

The needles of lodgepole pine are found in pairs, 3-7 cm in length, sometimes twisted, varying in color from light-yellowish green to dark green, with in tact bundle-sheath. Needle pairs run parallel rather than shaped in a "V" (Farrar, 1995). Koch (1996) found that the average length of air-dry needles was 5.34 mm which fits into the appropriate size range stated by Farrar, refer to Figure 16 (1995).

The bark of lodgepole pine is thin and orange to brown and grey in color (Farrar, 1995). These trees grow to 30 m in height, with a diameter at breast height of up to 60 cm, and live to an average age of 200 years. The stem of the tree tapers from base to crown, and the live crown is conical and located mostly at the apex of the stem (Farrar, 1995).

Jack pine is characterized by very distinct cones, which point away from the stem of the tree, and curve in towards the branch. These cones range from 3-7 cm long, are found in clusters of 2 or 3, and are serotinous (Farrar, 1995). Some non-serotinous cones are produced in the southern extent of the jack pine range (Forintek Canada Corp., 1994). Jack pine serotinous cones open in heat related events such as fire or direct sunlight, as seen in lodgepole pine. Cone scales in jack pine are thick and smooth, refer to Figure 17 (Farrar, 1995).

Needles of jack pine are paired and usually range from 2 to 4 cm long. Jack pine is the only short-needled pine in Canada with paired needles. These needles are usually straight but sometimes twisted, light green in color and spread apart to form a "V", with bundle sheath in tact, refer to Figure 16 (Farrar, 1995).

Jack pine bark is thin, reddish brown in color, and has scales that are flaky in a young tree, and grow into thicker irregular plate-like shapes in mature trees (Farrar, 1995). Jack pines grow up to 20 m in height, with a diameter at breast height of up to 30cm, and they live to 150 years of age on average. The stem tapers from base to crown (Farrar, 1995) and live crown is wide and irregular in shape (Forintek Canada Corp., 1994).

These characteristics are similar to what was found in the samples collected in this study.

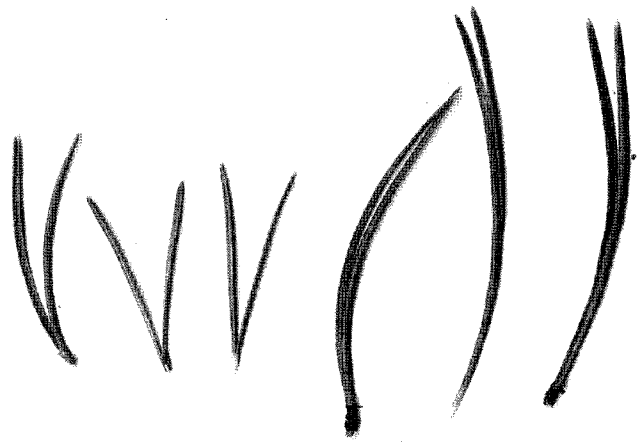

Figure 16: Needle forms for jack pine (left 3 needle pairs) and lodgepole pine (right 3 needle pairs).

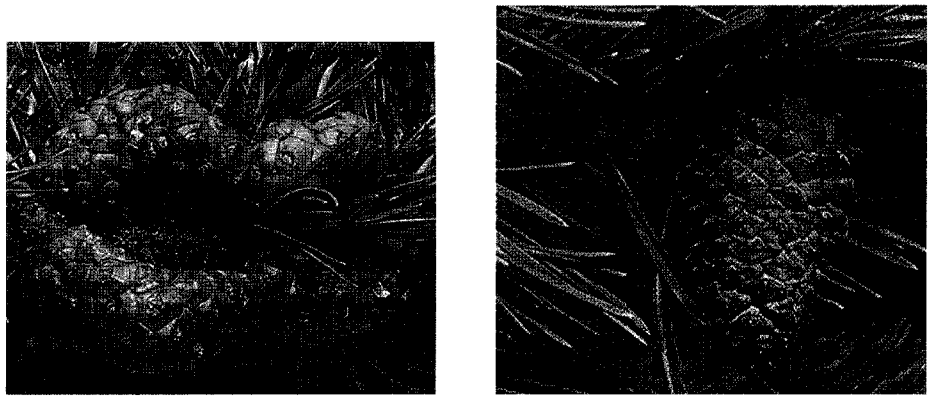

Figure 17: Cone forms for jack pine (left) and lodgepole pine (right) (from Farrar, 1995).

3.3 Experimental Design and Hypotheses

Gross morphological characteristics of each tree were measured, including: cone length and orientation, and needle length and position. Tree height and diameter at breast height (DBH) were recorded at time of sampling. These phenotypic characteristics were compared among the samples.

Thirty samples of pure lodgepole pine, 30 of pure jack pine, and 30 potential hybrid samples were collected from the areas of Prince George, BC, Smoky Lake, AB, and Fort Nelson BC, respectively. Each site was chosen for its similarity in vegetation and moisture/nutrient regimes. Sites for lodgepole pine and jack pine were chosen as to be similar distances from the potential hybrid zone.

The cones, needles, and heights of jack pine and lodgepole pine were different enough between samples for field identification of each species. The most prominent difference was the cone orientation. The potential hybrids had a combination of the characteristics of both species, for example, an average needle length in between that of the short jack pine needles and long lodgepole pine needles.

Some within species variation was expected due to inevitable site variation, but by using averages of all samples collected, and by comparing species based on site index, an appropriate representation of each species was gained.

3.4 Methodology

All sample trees were measured for gross morphology. Data was analyzed by comparing characteristics in order to establish trends and interactions between variables. General linear multivariate models were used to test the statistical significance of the results and pairwise comparisons were preformed in order to compare each species group with respect to each characteristic variable using SPSS version for Windows. Also, cluster analysis was preformed to establish sample groups that were the most similar to each other based on the characteristics identified.

3.4.1 Needles

Needle pairs were measured for length (dashed line on Figure 18) and width of "V" formation (solid line on Figure 18) using electronic calipers. A ratio of needle V width over needle length was used to compare samples; the width of the V is dependant on the length of the needles. Five needle pairs per sample were measured to obtain average dimensions; 150 needles per sampling area were measured in total.

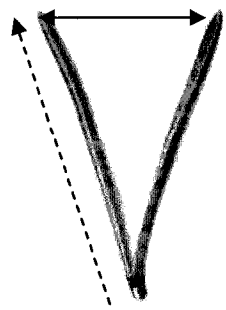

Figure 18: Needle Measurements

3.4.2 Cones

Cones were measured for length; curvature of the cones was noted for comparison. Cone length was measured from the tip of the cone to the base where the cone met the branch, not including any cone curvature (dashed line on Figure 19). Measurements of cone height from the branch (solid line on Figure 19) and length along the branch (dotted line on Figure 19) were also recorded in order to calculate the angle that the cone formed with the branch (Figure 19). Cone curvature was given a qualitative descriptor; slightly curved, moderately curved, largely curved, or not curved. An average of two cones per sample were measured depending on availability at time of sampling. In total, approximately 60 cones per sampling area were used to calculate dimensions.

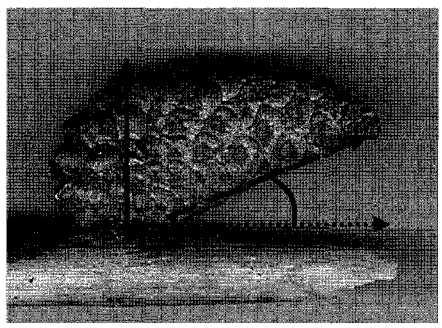

Figure 19: Cone Measurements

3.4.3 Height, DBH, and Age

Tree height was measured using a clinometer and measuring tape, and tree diameter at breast height was measured using a DBH tape. Age was determined within a 5 year error by counting the growth rings of each sample. Tree height, DBH and age were used to calculate growth rates of each tree sampled. Using growth rate instead of absolute height and DBH values allows comparison of various sites by factoring in stand age.

3.5 Results

3.5.1 Needles:

Results of mean length, V width, and range of lengths and V widths found are shown in Table 5. Figure 20 shows the average ratios (V width / needle length) for each sample group classified by sampling area.

Table 5: High and low range needle measurements displaying the variation within and among species.

	jack pine	lodgepole pine	hybrid pine
Avg. needle length (cm)	3.015	5.397	4.107
Avg. needle V width (cm)	1.457	0.986	1.372
Length min. value (cm)	1.900	2.900	2.700
Length max. value (cm)	4.700	10.600	6.200
V width min. value (cm)	0.200	0.000	0.100
V width max. value (cm)	4.000	3.900	2.900
Number of samples	150	150	150

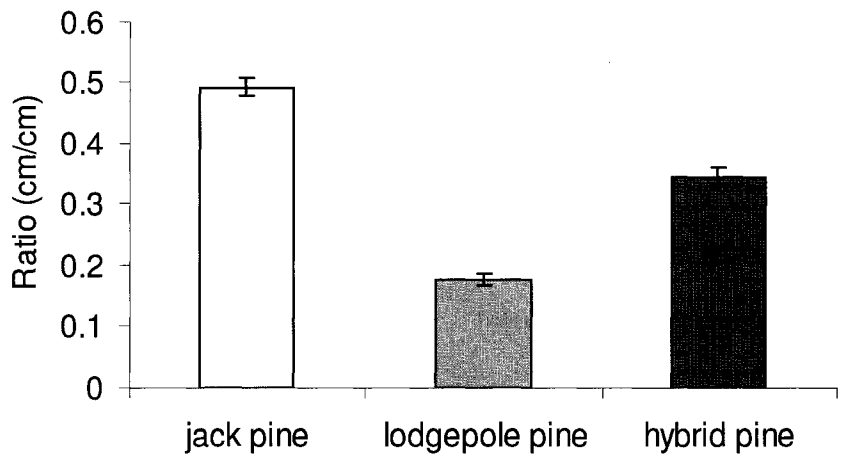

Figure 20: Average ratio of needle V width over needle length for each species/ sample group by area with standard error bars.

Figure 20 shows significant differences among the species based on the ratio of needle V width / needle length. Jack pine needles were characteristically much shorter and split in a wider "V" than lodgepole pine needles which are longer and oriented in a much tighter "V" formation. The hybrid pine needle measurements were found to be in between those of the other species, moderate in length and "V" width.

3.5.2 Cones

Table 6: Average cone angle for each species/sample group. Angles represent the number of degrees that the cone deviated from the branch. Negative values indicate that the cone curved over the branch to the opposite side from which it began growing.

	Jack Pine	Lodgepole Pine	Hybrid Pine
Mean Cone Angle (°) by sample area	-4.26	57.88	47.67
Number of samples	77	52	66

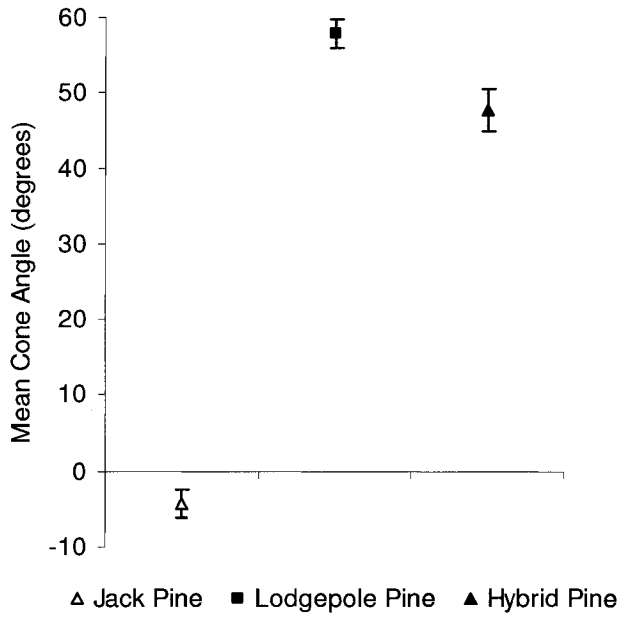

Figure 21a: Mean cone angle for all species groups according to sampling area with standard error bars.

Table 6 and Figure 21a display mean cone angles. The table gives a comparison between the cone angle averages calculated. Figure 21a shows that mean cone angles are lowest in jack pine and highest in lodgepole pine. Hybrid pine samples have intermediate cone angles. There are significant differences between species groups indicated by the standard error bars that are present.

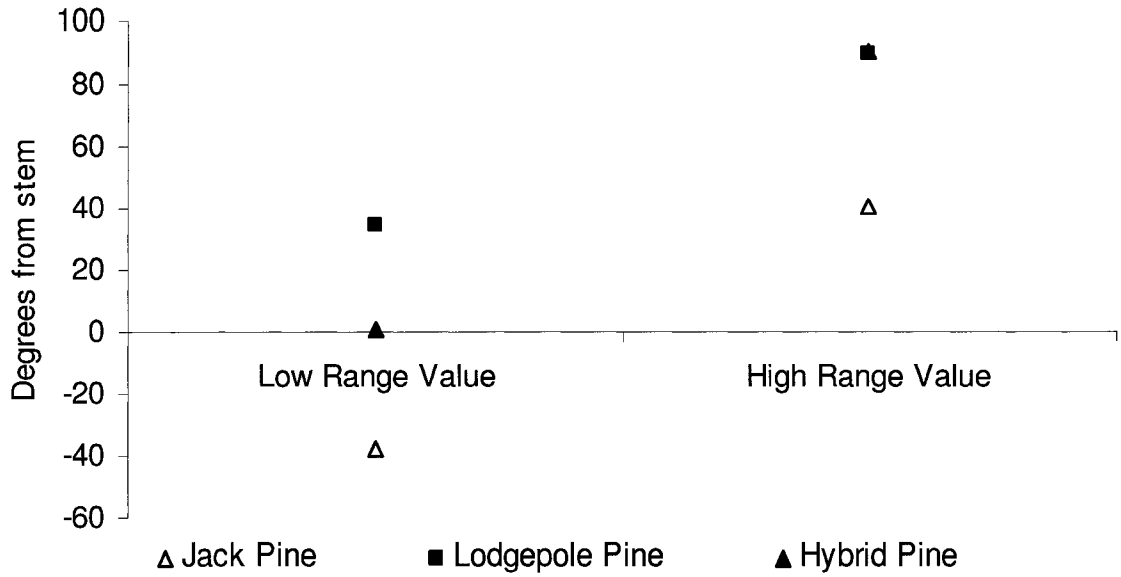

Figure 21b: The range in angles that the cones form with the branch for each species/sample group according to sample area. Low range value is the smallest or most negative angle observed, and high range value refers to the largest angle observed in that sample group. Negative values indicate that the cone curved over the branch to the opposite side from which it began growing.

It was observed that generally the lower or more negative the cone angle value, the more curvature the cone displayed. As seen in Figure 21b, jack pine cones tend to have lower angle values, than the other species groups, in both the high-range (highest angles found) and low-range (lowest angles found) categories. The lower the cone angle value, the closer the cone tip is to the branch. Clearly there is greater within "species" variability for the hybrid samples as compared to the jack pine or lodgepole pine samples for cone angle as a trait.

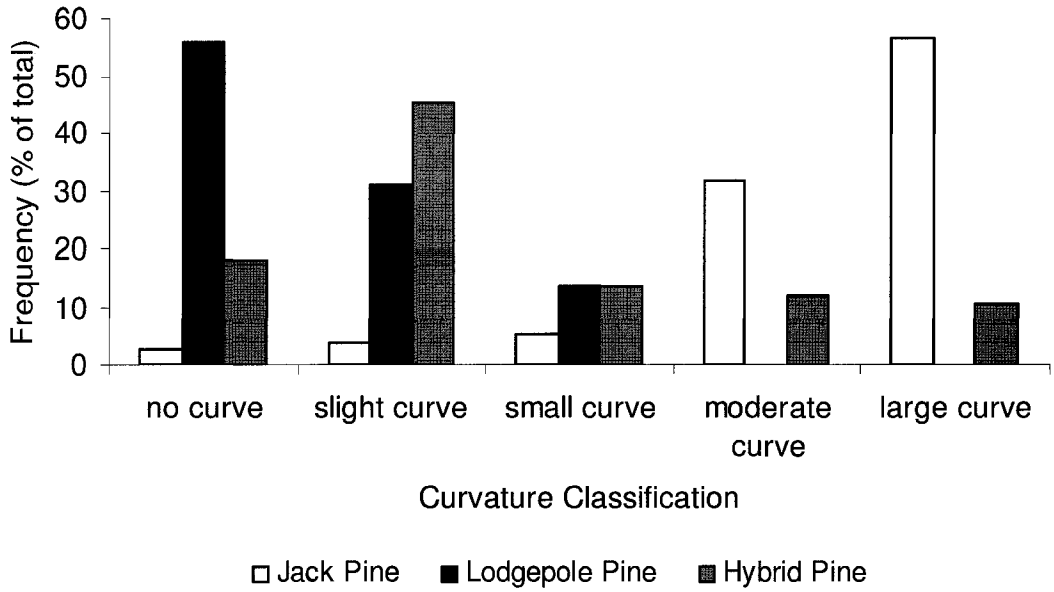

Figure 22: Qualitative classification of cones based on observed curvature.

Cones were classified based on comparative observation. As seen in Figure 22, less curvature was observed in lodgepole pine cones than in jack pine cones. Hybrid cones have high variability in cone curvature, with the highest percentage of cones being "slightly curved". As this is a purely qualitative observation it does not have statistical significance, but when added to the quantitative results, may illustrate a clearer picture of the differences between species groups.

Table 7: Cone length averages and range of variation in mm.

	Jack Pine	Lodgepole Pine	Hybrid Pine
Avg. Length (mm)	36.55	36.2	42.87
Shortest cone (mm)	21	23	29
Longest cone (mm)	58.5	44.5	54.5
Number of samples	77	52	66
Cone length SE	1.260	1.135	1.029

Average cone lengths and variation in cone lengths are shown in Table 7. The average lengths were similar among species, hybrids display longer cones on average, possibly due to factors that will be discussed in Section 2.5. Jack pine cones had a higher range of cone length values than lodgepole pine.

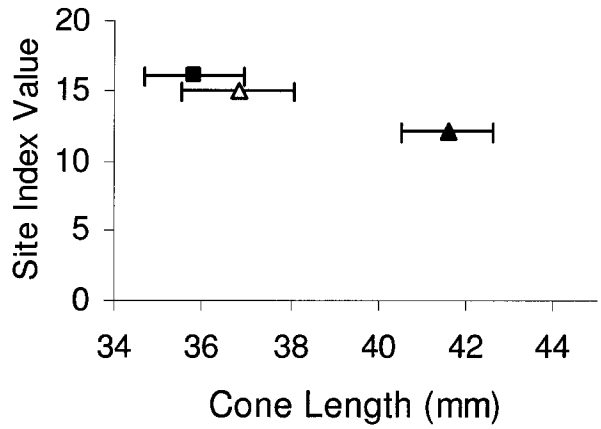

Figure 23: Site index as a function of cone length for sample groups according to sampling area
Δ Jack pine ■ Lodgepole pine ▲ Hybrid pine.

Figure 23 shows the effect that site index has on cone length. Lodgepole pine and jack pine site indices and cone lengths are similar; hybrid average cone length is significantly greater even though the site index value is the lowest for these samples.

Figure 24: Cone length as a function of Age Class

Figure 24 shows cone length as a function of age class. Age classes were defined and given a representative number from 1 – 7. Age class 1 represents trees aged 20-39, age class

2 represents trees aged 40-59, age class 3 = trees aged 60-79, age class 4 = trees aged 80-99, age class 5 = trees aged 100-119, and age class 6 = trees aged 120-139. Jack pine samples fall into the lower age classes, while lodgepole pine samples occupy the older age classes. Hybrid samples range in ages and cone lengths. Even though jack pine samples are younger, their cones demonstrate a similar average length, and a high-range length value greater than lodgepole pine. Hybrid cones on average are longer than the other species groups, but samples are not necessarily from older age classes. Focusing only on age class 3, where all three species groups have representative samples, and based on Figure 24, lodgepole pine cones are shorter than the other species groups while hybrid cones are the longest. Cone length statistics are shown in Table 9.

3.5.3 Height, DBH, and Age

Table 8: Average height, DBH and age values for the given sample groups, and range in height, DBH and age.

	Jack Pine	Lodgepole Pine	Hybrid Pine
Avg. height (m)	15.34	22.46	17.86
Shortest Tree (m)	10.1	18.58	11.64
Tallest Tree (m)	23.06	27.04	22.9
Avg. DBH (cm)	24.94	22.52	23.92
Smallest DBH (cm)	16.1	16.3	17
Largest DBH (cm)	36.6	30.3	41.3
Avg. Age (yrs)	53.31	83.18	68.59
Youngest (yrs)	35	62	44
Oldest (yrs)	69	140	120
Number of samples	30	30	30

Table 8 displays the average height, DBH, and age for each species group according to sampling area, as well as the range in these values found within each group. As shown, jack pine has the shortest average height, but also the shortest range in height among all samples. Lodgepole pine has the tallest average height and the tallest range among samples. This correlates well to the age information, which shows that the lodgepole pine trees were the

oldest of all trees sampled and the jack pine trees were the youngest. Therefore variation in height can be explained by age differences. In order to compare samples for height differences without having an age bias, growth rate (height divided by age) was used for further comparison.

The same issue exists when dealing with DBH, as intuitively, DBH changes with the age of the tree. Therefore, growth rate (DBH divided by age) is used for comparing DBH between samples to eliminate any age biases. Observations of DBH suggest that jack pine has that greatest average diameter, and lodgepole pine has the smallest average diameter. Hybrid samples show the widest range in DBH.

Figure 25 displays average growth rates for all species groups, and Figure 26 shows how the differences in growth rates for height and DBH might be explained by site index differences.

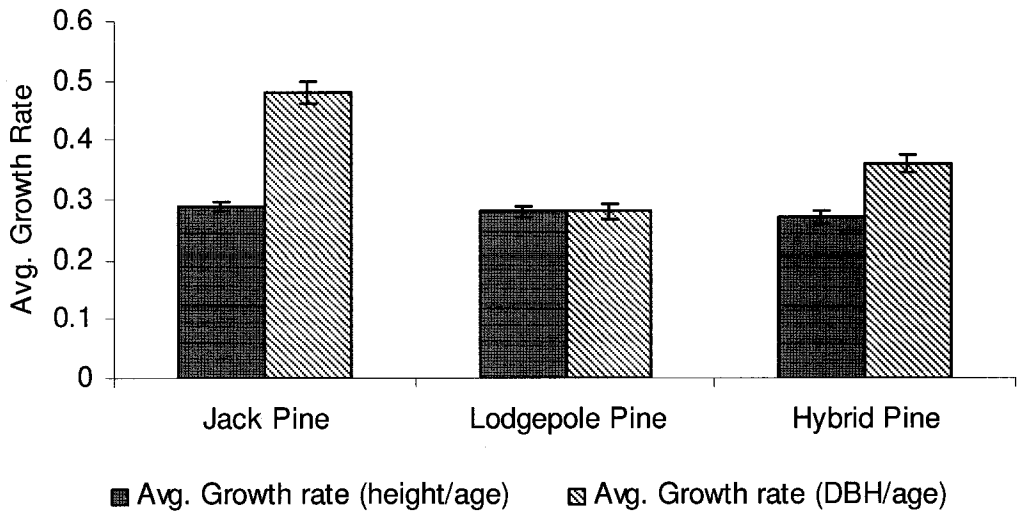

Figure 25: Growth rates for all sample groups according to area.

In Figure 25 there is no significant difference shown among species' growth rates with respect to height.

Growth rates for DBH show a significant difference between all three species groups. Jack pine has the fastest growth rate based on DBH, lodgepole pine has the slowest growth rate, and the hybrid samples display an average growth rate in between those of the pure species. Further investigation is required to help explain these growth rate differences. Comparing DBH growth rate to site indices may offer an explanation.

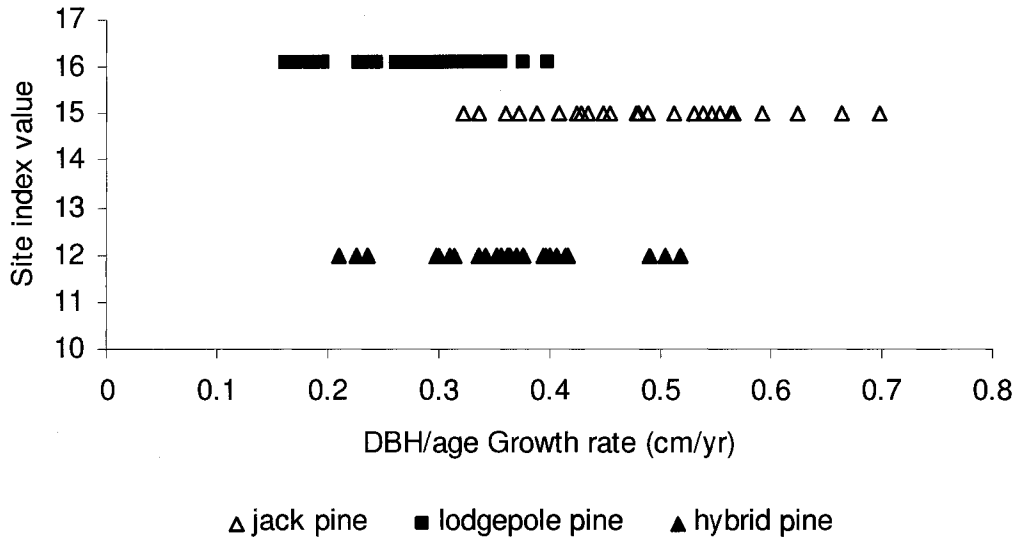

Figure 26: Site index as a function of Growth rate (DBH/age)

Figure 26 shows that DBH growth seems relatively unaffected by differences in site index value for these sample groups. Jack pine displays the highest rate of DBH growth, and lodgepole pine shows the lowest growth rate. The average growth rate for hybrids is moderate among these samples, even though they were found on sites with the lowest index potential.

3.5.4 Statistical Analysis

Table 9: Pairwise Comparisons for morphology. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
needle_w_l	1	2	.319(*)	.033	.000	.253	.385
		3	.081(*)	.033	.017	.015	.146
		4	.006	.057	.920	-.109	.120
		5	.265(*)	.040	.000	.186	.345
	2	1	-.319(*)	.033	.000	-.385	-.253
		3	-.238(*)	.036	.000	-.309	-.167
		4	-.313(*)	.059	.000	-.431	-.196
		5	-.054	.042	.206	-.138	.030
	3	1	-.081(*)	.033	.017	-.146	-.015
		2	.238(*)	.036	.000	.167	.309
		4	-.075	.059	.207	-.193	.042
		5	.185(*)	.042	.000	.101	.268
	4	1	-.006	.057	.920	-.120	.109
		2	.313(*)	.059	.000	.196	.431
		3	.075	.059	.207	-.042	.193
		5	.260(*)	.063	.000	.134	.385
	5	1	-.265(*)	.040	.000	-.345	-.186
		2	.054	.042	.206	-.030	.138
		3	-.185(*)	.042	.000	-.268	-.101
		4	-.260(*)	.063	.000	-.385	-.134
cone length (mm)	1	2	3.071	1.759	.085	-.438	6.580
		3	-3.431	1.759	.055	-6.940	.078
		4	8.680(*)	3.065	.006	2.568	14.793
		5	-2.911	2.129	.176	-7.158	1.336
	2	1	-3.071	1.759	.085	-6.580	.438
		3	-6.502(*)	1.897	.001	-10.286	-2.718
		4	5.610	3.146	.079	-.665	11.884
		5	-5.982(*)	2.245	.010	-10.459	-1.505
	3	1	3.431	1.759	.055	-.078	6.940
		2	6.502(*)	1.897	.001	2.718	10.286
		4	12.112(*)	3.146	.000	5.837	18.386
		5	.520	2.245	.818	-3.957	4.997
	4	1	-8.680(*)	3.065	.006	-14.793	-2.568
		2	-5.610	3.146	.079	-11.884	.665
		3	-12.112(*)	3.146	.000	-18.386	-5.837
		5	-11.592(*)	3.367	.001	-18.307	-4.876
	5	1	2.911	2.129	.176	-1.336	7.158

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
		2	5.982(*)	2.245	.010	1.505	10.459
		3	-.520	2.245	.818	-4.997	3.957
		4	11.592(*)	3.367	.001	4.876	18.307
Height/Age	1	2	-.005	.016	.770	-.036	.027
		3	.017	.016	.282	-.014	.049
		4	-.027	.028	.325	-.082	.028
		5	.020	.019	.291	-.018	.059
	2	1	.005	.016	.770	-.027	.036
		3	.022	.017	.205	-.012	.056
		4	-.023	.028	.425	-.079	.034
		5	.025	.020	.219	-.015	.065
	3	1	-.017	.016	.282	-.049	.014
		2	-.022	.017	.205	-.056	.012
		4	-.045	.028	.120	-.101	.012
		5	.003	.020	.874	-.037	.044
	4	1	.027	.028	.325	-.028	.082
		2	.023	.028	.425	-.034	.079
		3	.045	.028	.120	-.012	.101
		5	.048	.030	.120	-.013	.108
	5	1	-.020	.019	.291	-.059	.018
		2	-.025	.020	.219	-.065	.015
		3	-.003	.020	.874	-.044	.037
		4	-.048	.030	.120	-.108	.013
DBH/Age	1	2	.187(*)	.026	.000	.135	.238
		3	.153(*)	.026	.000	.102	.204
		4	.039	.045	.389	-.050	.128
		5	.070(*)	.031	.027	.008	.132
	2	1	-.187(*)	.026	.000	-.238	-.135
		3	-.033	.028	.235	-.088	.022
		4	-.148(*)	.046	.002	-.239	-.057
		5	-.116(*)	.033	.001	-.182	-.051
	3	1	-.153(*)	.026	.000	-.204	-.102
		2	.033	.028	.235	-.022	.088
		4	-.115(*)	.046	.014	-.206	-.024
		5	-.083(*)	.033	.013	-.149	-.018
	4	1	-.039	.045	.389	-.128	.050
		2	.148(*)	.046	.002	.057	.239
		3	.115(*)	.046	.014	.024	.206
		5	.031	.049	.524	-.066	.129
	5	1	-.070(*)	.031	.027	-.132	-.008
		2	.116(*)	.033	.001	.051	.182
		3	.083(*)	.033	.013	.018	.149
		4	-.031	.049	.524	-.129	.066
cone angle	1	2	-61.269(*)	4.394	.000	-70.032	-52.506

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
		3	-44.877(*)	4.394	.000	-53.639	-36.114
		4	7.630	7.654	.322	-7.635	22.896
		5	-64.680(*)	5.318	.000	-75.286	-54.073
	2	1	61.269(*)	4.394	.000	52.506	70.032
		3	16.392(*)	4.738	.001	6.943	25.841
		4	68.899(*)	7.857	.000	53.229	84.569
		5	-3.411	5.606	.545	-14.591	7.770
	3	1	44.877(*)	4.394	.000	36.114	53.639
		2	-16.392(*)	4.738	.001	-25.841	-6.943
		4	52.507(*)	7.857	.000	36.837	68.177
		5	-19.803(*)	5.606	.001	-30.983	-8.623
	4	1	-7.630	7.654	.322	-22.896	7.635
		2	-68.899(*)	7.857	.000	-84.569	-53.229
		3	-52.507(*)	7.857	.000	-68.177	-36.837
		5	-72.310(*)	8.409	.000	-89.081	-55.539
	5	1	64.680(*)	5.318	.000	54.073	75.286
		2	3.411	5.606	.545	-7.770	14.591
		3	19.803(*)	5.606	.001	8.623	30.983
		4	72.310(*)	8.409	.000	55.539	89.081

Based on estimated marginal means

* The mean difference is significant at the .05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Cluster Analysis:

Table 10a: Final Cluster Centers for Morphology

	Cluster		
	1	2	3
Needle_w_l	.3923	.2319	.4846
cone length (mm)	41.0	37.7	37.4
Height/Age	.2650	.2844	.2941
DBH/Age	.3580	.3366	.4842
Cone angle	26.0606	60.6495	-7.8530

Table 10b: Distances between Final Cluster Centers for morphology

Cluster	1	2	3
1		34.750	34.099
2	34.750		68.503
3	34.099	68.503	

Table 10c: Number of Cases in each Cluster for morphology

Cluster	1	17.000
	2	32.000
	3	26.000
Valid		75.000
Missing		16.000

Further statistical data can be found in Appendix 3. The cluster analysis in Table 10a, b, and c, reveals that most jack pine samples fall into as cluster 3, while lodgepole pine samples were categorized as cluster 2 and the majority of hybrid samples were categorized as either cluster 1 or 2. A few jack pine samples fell into cluster 1; sample 5, 8, 10, and 25. Only sample 63, out of the hybrid samples, was identified as part of cluster 3. Samples 61, 62, 64, 65, 67, 68, 69, 71, 75, 76, 80, 81, and 90 fell into cluster 1; samples 66, 72, 74, 77, 78, 79, and 82 to 89 fell into cluster 2.

3.6 Discussion and Conclusions

3.6.1 Needle Characteristics

Needle V width and needle length are two characteristics that are identifiably different among species of pines in this study (Table 5), and supports work by Farrar (1995), and Koch (1996). Wheeler and Guries (1987) used Lubischew's coefficient of determination in order to evaluate how effective a particular morphological trait was at distinguishing between species. The greater the coefficient, K, the more "discriminating power" the trait has; $K = (X_A-X_B)^2/2 * (s_A*s_B)$ where X is the mean of a certain characteristic in species A and B, and s is the standard deviation of that characteristic in species A and B (Christensen, 2003). Needle length was used in the study by Wheeler and Guries as one of the traits that had significant ability to distinguish between species. Other traits that are also used in the study by Wheeler and Guries (1987) include cone curvature, angle of cone attachment, and cone

length. Each of these traits allows for supportive species identification, and when observed in combination allows for accurate assessment of species type. The information provided by Lubischew's coeffient of determination for the characteristics analyzed by Wheeler and Guries indicates that cone angle of attachment and needle length, among others, are very useful for discriminating between hybrids and pure pine species. This provides support for the use of these characteristics in this study.

To add to this, needle V width used as a ratio with needle length provides further support in species identification. As seen in the results in Figure 20, needle V width/length produced a significant difference among all species groups (jack pine, lodgepole pine, and hybrids). Figure 20 shows that the ratio for needle V width/length is highest in jack pine, lowest in lodgepole pine, and intermediate in hybrids, indicating that this trait was modified in the hybrids due to genetic recombination to produce intermediate needle formations. In further support that the pure species and hybrids show variation, a pairwise comparison between species groups revealed that the mean differences between all three main species groups, when compared to one another, were statistically different for needle width/length (Table 9). Species groups 4 and 5 showed that jack pine or hybrid samples were not significantly different from group 4, and lodgepole pine samples were not statistically different from group 5.

3.6.2 Cone Characteristics

Cone curvature, angle of cone attachment, and cone length are also morphological characteristics that distinguish between species (Wheeler and Guries, 1987). In this study, significant differences in cone angles were found among all species groups; variation in

angle, i.e. the total range within all samples, was also found to be unique for jack pine and lodgepole pine. This indicates that cone angle is probably one of the largest differences in morphology between these two pines. In fact, according to Wheeler and Guries (1987), the coefficient of determination for angle of cone attachment is one of the most discriminating factors between jack pine and lodgepole pine. In their study, lodgepole pine cone angles were an average of 120° +/- 22.6, jack pine cone angles were 16.5° +/- 29.5 and hybrid cone angles were an average of 52.8° +/- 41.0. The same pattern was found in this study, lodgepole pine angles of cone attachment were large, jack pine angles are small, hybrid angles are intermediate and contain the most variability. The pairwise comparison revealed that cone angles were significantly different among all three main species groups (Table 9). As seen in the needle V width/ length characteristic, species group 4 is not significantly different from jack pines, and species group 5 is not significantly different from lodgepole pines.

The result suggests that hybrid pine cone angles are a characteristic feature that may be genetically controlled and may have been influenced by both pure species, forming an intermediate, genetically recombined, characteristic cone angle. This finding parallels needle V width/length ratio, which also suggested a recombined intermediate characteristic.

Wheeler and Guries (1987) described cone curvature in a quantitative way by measuring the arc of the curve. In this study, cone curvature was not investigated to its full extent, but simply given a qualitative value based on comparative observation. As shown in Figure 22, lodgepole pine cones sampled were much straighter than jack pine cones. Hybrid cones formed intermediate curves and contained the highest variability, similar to the cone's angle of attachment as discussed above, providing supportive evidence of "intermediacy" in

the hybrids studied, as compared to the pure species samples of jack pine or lodgepole pine. Since the cone angle and curvature were found to be so different among these pines, cones can provide a very important distinguishing feature for field identification of hybridization.

Cone length is another characteristic that has been identified as a possible distinguishing feature among the pine species. The average cone length was similar for jack pine and lodgepole pine in this study, but hybrid cones were longer on average (Table 7). Variability in cone length was fairly consistent throughout the species groups. The statistical pairwise comparison in Table 9 revealed that the cone length for hybrids was significantly different from jack pine and lodgepole pine, but the pure species were not significantly different from each other, indicating that cone length does not act as a characteristic displaying intermediacy. However, this result does assist in distinguishing hybrids from pure species within these samples.

3.6.3 Site Considerations

Characteristics mentioned such as needle V width/length, cone angle of attachment, and cone curvature may be unaffected by site, because there would be little advantage for a tree to develop these characteristics in one way or another with changes to its environment. Needle length on its own may be affected by light exposure on some sites, however, when combined as a ratio needle with V width, the value represents total needle development rather than just growth. Alternatively, cone length may be affected by site conditions.

For this reason, cone length was investigated as a function of site index in Figure 23, and as a function of age class in Figure 24. The expected trend, the greater the site index value the greater the cone length, was not observed in these figures. Hybrids, with the lowest

site index value, have the longest cones on average. Therefore differences in cone length can not be explained by site index variation; the expected outcome, that cones may grow larger on good sites to increase reproduction, was not observed. Age class did not have a significant impact on cone length. By selecting only age class 3, where all species groups were represented, comparing cone length showed that hybrid cones are longer than both jack pine and lodgepole pine; the same outcome as comparing overall averages. Wheeler and Guries (1987) identified cone length as a possible distinguishing feature between jack pine, lodgepole pine, and hybrids, however did not perform full analysis using this trait because it scored fairly low as a test for Lubischew's coefficient of determination. For the purpose of this study, cone length provided distinction between hybrids and lodgepole pine, but not between jack pine and any main species group or as a representation of intermediacy between the pure species.

3.6.4 Growth Rate Characteristics – Height/Age and DBH/Age

The final two characteristics that were used to identify morphological traits among pines in this study were height and DBH used as a growth rate ratio over age. Since age range varied among the species sampling groups and height and DBH are directly related to age, it was necessary to compare the groups based on growth rates. Table 8 shows the measured values of DBH, height, and age before ratio calculations.

Figure 25 shows that there are no significant differences in height growth rate for the three species groups by a pairwise comparison; Table 9 shows no significant difference between species groups for the height/age variable. Therefore growth rate represented as

height/age is similar between species groups, and is not a valuable tool for species and hybrid identification.

Contrary to this, the growth rate for DBH did show statistically significant variation between species, both in Figure 25 and in the pairwise comparison (Table 9). Since growth rate is heavily dependent on site conditions, it is necessary to discuss DBH/age as a function of site index value. Figure 26 shows that lodgepole pine has the greatest site index value, which would normally coordinate with the highest growth rate. However, jack pine demonstrates the highest growth rate while lodgepole pine has the lowest growth rate. Hybrids demonstrate the lowest site index value but have an intermediate growth rate. Therefore, the differences in growth rate can not be attributed to site index, or site potential in this study. The pairwise comparison for DBH/age (Table 9) shows that there are significant differences between pure species, and between jack pine and hybrids, but not between lodgepole pine and hybrids. These statistical results indicate that hybrids may be more similar to lodgepole pines with respect to DBH growth rate.

Although there is sufficient analysis to support the relevance of growth rate according to DBH in this study, it would be prudent to investigate it further before identifying it as a strong distinguishing feature between species because of its "heavy" reliance on site. From preliminary work on DBH growth rate, it has the potential to act as a distinguishing feature to identify hybridization between jack pine and lodgepole pine by intermediacy, similar to needle V width/length, cone angle, and cone curvature.

3.6.5 Cluster Analysis

Table 10a, b, and c show the results from the cluster analysis; the values for the cluster centers, distances between cluster centers, and the number of cases in each cluster. Three clusters were formed from samples that contained values for all the input variables. Fifteen samples were missing values and therefore not included in the cluster analysis. Individual samples included in each cluster can be found in Appendix 3 along with an ANOVA table for the cluster analysis. Based on the ANOVA output, needle V width/length, DBH/age, and cone angle were significant variables in distinguishing between clusters. Cone length and height/age were not significant; this is not surprising considering their ambiguity in distinguishing between species in previous analysis. The samples were grouped in the cluster analysis to produce the most variability between clusters. So, based on the output, most jack pine samples varied significantly from the lodgepole pine and hybrid samples according to morphological traits that were input (forming cluster 3). Lodgepole pine samples varied from jack pine samples and were grouped into cluster 2. Hybrid samples however, did not form a whole distinct cluster. Approximately half the hybrid samples formed their own cluster (cluster 1), and the other half remained in cluster 2 with the lodgepole pine samples, indicating that there was more variability within the hybrids than between some of the hybrids and the lodgepole pine samples. This could be due to the fact that samples 81- 90 were found to have unique maternal and paternal haplotypes even though resembling hybrids for some traits, such as cone angle and curvature. Also it indicates that these samples were probably not first generation (F1) hybrids, and more likely a back-cross of hybrid and lodgepole pine, causing most of the morphological jack pine traits and genetic information to be lost.

Overall, the cluster analysis does display a large amount of between species variation based on the morphological traits identified, therefore allowing for a large amount of distinction between species groups.

3.6.6 Conclusions

It is important to identify morphological characteristics between species and hybrids for rapid field identification of these trees. During timber harvesting, forest management, or research of the areas containing hybrids, it is valuable to be able to quickly and accurately identify species and hybrids.

Several morphological characteristics provide significant differences between the three species groups identified in this study; jack pine, lodgepole pine, and hybrids. These differences provide distinction between the species groups and allow for field identification, as well as support the concept of hybridization. Characteristics that have been identified to distinguish between species groups include: needle V width/length, cone angle of attachment, cone curvature, cone length, and DBH/age. These characteristics not only distinguish between species but also show that the hybrids are intermediate to the pure species, with the exception of cone length. Cone length was also identified as a distinguishing characteristic, however does not identify intermediacy for hybrids. Intermediacy is an important factor to consider because it indicates that the hybrids are not only different from the pure species, but contain a combination of their genetic traits. Therefore hybrids are in fact a "mixture" of both pure species.

Identifying the difference between jack pine, lodgepole pine, and hybrids of these species in the field is possible based on the characteristics investigated in this study, which

addresses the first objective of the morphological traits. Some characteristics that had been used to identify hybrids in Alberta and N.W.T. (Wheeler and Guries, 1987, and Critchfield, 1985) were used in this study to identify hybrids in British Columbia. This study successfully identified characteristics among species with strong discriminatory abilities versus characteristics that were weaker or not useful for distinction. Characteristics that are most useful for distinction between species are needle V width/length and cone angle, which not only distinguished between pure species and hybrids, but also displayed intermediacy in hybrids. Furthermore, growth rate by DBH showed the ability to distinguish between jack pine and hybrids, and indicated that hybrid sample growth may be more like that of lodgepole pine than jack pine in this study.

Sources of error exist within the morphological study that may have contributed to some of the variability in the results. Since the trees sampled were of a mature age, collecting needles and cones from all over the live crowns was difficult. This means that needles and cones were primarily collected from the bottom half of the live crown which could have affected average overall size of the needles or cones. It is also possible that because trees were collected from different sites they were subject to varying conditions other than the differences explored in this study.

Many characteristics were used in other studies for identification of hybrids that were not used in this study. In order to build on the information gathered here, and support the conclusions drawn, these additional characteristics could be studied in jack pines, lodgepole pines, and their hybrids for their ability to distinguish species groups. Wheeler and Guries (1987) used leaf serrations, prickle, and seed coat mottling among others, as distinguishing traits with successful results; Christensen and Dar (2003) used the number of stomata on the

adaxial surface as well as other traits for comparisons. Another comparative study in *Juniperus* by Adams (2003), used leaf margins (entire vs. serrate), leaf thickness, and length of stomatal apparatus to distinguish between various species, all of which could be used for the pine species in this study.

3.7 References

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Chapter 4: Fibre and Cell Analysis of Jack Pine, Lodgepole Pine, and their Hybrids

4.1 Chapter Objectives

Since processing and manufacturing of wood and wood products requires specific adjustments for variations in wood quality and therefore species, it is important to know where these variations occur and which areas of British Columbia are providing which types of wood.

Interactions between various wood traits or fibre traits are explored in this chapter to determine where possible relationships occur or from where they may originate. This aids in comparison between species as it was previously determined in Chapter 3 that each sampling area varied slightly in age, growth rate, and site index.

The objective of this chapter was to compare each species group based on wood and fibre traits in order to identify if there were any significant differences between lodgepole pine and jack pine, and their hybrids. Using the obvious morphological differences between species and hybrids, samples were classified according to sampling area. Variation in wood and fibre traits was compared in these same classifications to determine if these traits can be used as distinguishing features between jack pine, lodgepole pine, and their hybrids.

Another objective of this chapter was to identify hybrid characteristics closely related to jack pine, and those characteristics that are more closely related to lodgepole pine. Inferences can be made about the inheritance of the traits and tree lineage within the hybrid sampling area. By identifying the differences between species groups with regard to wood and fibre traits, and comparing these results to genetic outcomes of Chapter 2, it was hoped that the wood and/or fibre characteristics may then be used to predict hybridization.

4.2 Wood and Fibre Characteristics

As various wood and fibre characteristics are investigated, it is important to keep in mind that three main factors contribute to variation in these traits, and that a difference in characteristic between species can be due to one of these factors, or more probably due to all three of them in combination. These factors are: 1) the changes in wood cells and fibres as they age; 2) the genetic influence of the parental trees; and, 3) the environmental influences that affect the tree growth (Panshin and de Zeeuw, 1980).

Lodgepole pine has varying angles of grain spirality causing a cross-grained effect among the tracheids (Koch, 1996), but it is generally considered a straight-grained wood. Sapwood in lodgepole pine is white to yellow in color, heartwood is yellow to brown. The wood is fairly light weight and somewhat weak in bending and when compressing end-to-end. Lodgepole pine is susceptible to decay when conditions are favorable (Alden, 1997). Similar to lodgepole pine in most characteristics, jack pine possesses a few slight differences in its wood properties and appearance. Jack pine sapwood is almost white in color, while the heartwood is light brown or orange. The wood is resinous and moderately light weight with low shrinkage properties for drying, and has limited durability. It has low bending and compressive strength. Jack pine, like lodgepole pine, is also susceptible to decay when conditions are favorable (Alden, 1997).

Lodgepole pine and jack pine woods are characterized by growth rings that show an abrupt transition from earlywood (light in color) to latewood (dark in color). Even though the transition between growth rings is equally abrupt in both species, a differentiation is possible in the amounts of both earlywood and latewood present between species. The ratio of earlywood to latewood can identify a wood as high or low quality for processing or

manufacturing depending on the characteristics desired in the product. Earlywood (EW) and latewood (LW) possess very different characteristics in hard pines, which is why the ratio of EW:LW can provide important information about a species. In earlywood, the longitudinal tracheids usually have a larger diameter in the radial direction and thinner cell walls. However, in latewood, tracheids have thick walls and are almost flattened radially. This change in cell shape is due to the seasonal growth changes; large earlywood tracheids are formed during active internodal elongation, when the growth hormone, auxin, is being produced at a maximum. As auxin production declines, elongation slows down and cell diameters decrease yielding latewood tracheids. The abrupt transition from earlywood to latewood characteristic in pines forms a defined tangential band of latewood with thick flattened cells, and suggests that auxin production weans quickly. This latewood is therefore much denser than the earlywood; profiles of wood from pith to bark not only show increases in density as the wood matures, but also show immense variation in density within rings (Panshin and de Zeeuw, 1980).

Juvenile wood in lodgepole pine is located near the pith and is less prominent than in other conifers in North America with the transition to mature wood gradual in increasing tracheid length and decreasing in microfibril angle (Koch, 1996). There are significant cell and fibre level differences between juvenile wood and mature wood in all species. Juvenile wood is a column of inferior wood that is formed from prolonged influences of the apical meristems during wood formation. This portion of the tree is considered to be substandard from a wood quality point of view because it is prone to extensive warping when dried as lumber due to large longitudinal shrinkage (Panshin and de Zeeuw, 1980). Juvenile wood contains shorter fibres that have greater microfibril angles, and smaller tangential cell

diameters (Barbour, 2004). Juvenile wood is also less dense in most cases due to relatively fast growth rates, and contains shorter fibres which confine juvenile wood to specific paper products. In hard pines, it is characteristic in most cases that density will increase from pith to bark (Panshin and de Zeeuw, 1980).

Average density for lodgepole pine has been previously calculated as 39 lbs/cu.ft. or 0.625 g/cm^3 for green wood, and 27 lbs/cu.ft. or 0.43 g/cm^3 for oven dried wood (Isenberg, 1951). Koch (1996) stated that average specific gravity based on green volume and oven dried weight was 0.41 g/cm^3. Recorded density is 50 lbs./cu.ft or 0.801 g/cm^3 for jack pine green wood and 29 lbs./cu.ft. or 0.465 g/cm^3 for oven dried wood (Isenberg, 1951), which is higher than that of lodgepole pine. In general terms, specific gravity is "...the amount of cell wall substance present in wood..." (Panshin and de Zeeuw, 1980), and has a direct linear relationship with density, which is the ratio of the weight of wood due to cell wall content, to the volume. Therefore, specific gravity and density yield the same information about a particular sample or species.

Density changes when the cell wall thickness changes or when cell dimensions change. Photosynthetic input controls the amount of cell wall material that is produced in the tree (Panshin and de Zeeuw, 1980). Cell wall thickness therefore has a linear relationship to density and has the potential to distinguish between species provided that site conditions and age of samples remain constant. As stated, density increases from pith to bark. It is therefore intuitive that cell wall thickness also increases from pith to bark. This is due to the increase in diameter of cell walls as the tree matures, with little increase in tracheid length (Panshin and de Zeeuw, 1980).

A wood quality trait is dependent on the end use of the product. Generally, density is considered to be the most important wood characteristic for identifying quality because it suggests thick walled, strong cells that will hold together under stresses such as bending or compression, which are common in building. Modulus of elasticity (MOE) quantifies the flexibility of wood under compression parallel to the grain. This characteristic can be estimated based on microfibril angle (MFA) or tracheid lengths, indicating that significant relationships exist between MOE, fibre length, and MFA (Panshin and de Zeeuw, 1980).

Lodgepole pine has varying fibre dimensions. Sharma and Potter (2000) determined fibre dimensions of 3.1 mm x 35-45 um and 23.0 mg/100 m for lodgepole pine fibres, where Koch (1996) calculated an average fibre length of 2.2 mm. This is a big difference in length, however, these measurements are dependent on the age of the wood measured, and the site conditions under which the samples were grown such as latitude, elevation, moisture and nutrient regime, as well as the measurement method. Jack pine fibre dimensions are recorded as 3.5 mm x 28-40 um and 18.0 mg/100 m by Sharma and Potter (2000). This fibre information can be used to distinguish desirable processing traits and correlate them based on species group. Fibre length is an important characteristic to consider because pulp products have different fibre requirements. Longer fibres produce increased bonding area between fibres which yields paper that is less likely to tear under stress (Panshin and de Zeeuw, 1980). The same strength standards would not be required for tissue as for writing paper. Fibre coarseness is the most important characteristic to consider for paper production.

Fibre coarseness is defined as the weight of a fibre per unit length (Robertson, 1998). This characteristic, like fibre length, is variable between species, age classes, and site conditions. Trees grown on poorer sites, or trees younger in age will generally have finer

fibres than others. Lodgepole pine is said to have coarser fibres on average than other species such as Engelmann spruce, white spruce, or subalpine fir (Pitts, 2001). Jack pine may have similar fibre coarseness to lodgepole pine, but coarseness will not be identical.

Fibre yield is important to consider from a quality and quantity point of view. The amount of pulp that can be derived from a species is a key part of processing, but it is also valuable to explore the quality of the fibres, in order to predict what products the pulp can be used for. Fibre quantity depends on the density of the wood, EW:LW ratios, the age of the tree, the health of the tree, and some others. Fibre quality depends on cell characteristics and the type of cells present (Panshin and de Zeeuw, 1980).

Another important characteristic for wood manufacturing and processing is microfibril angle (MFA). Microfibrils are long strands of polysaccharides found in woody plant cell walls. These are physically oriented at various angles depending on where they are found within the cell wall, and help to identify the strength of the wood. The angle is measured from the cell axis, and is referred to as microfibril angle (MFA). As the cell matures, the orientation of the microfibrils changes; giving rise to different characteristics for juvenile and mature wood. In general, juvenile wood has higher microfibril angles than mature wood; the decrease in angle as the cells mature is related to the growth of the cell (Panshin and de Zeeuw, 1980). MFA is directly related to fibre strength. Increases in MFA result in decreases in strength, thereby decreasing the strength of a paper product. Therefore, like fibre length, different standards are required for microfibril angle, depending on the end use of the product.

Each of the wood and fibre properties mentioned plays a key role in determining wood quality, and therefore affects the manufacturing and processing procedures and end products.

It is important to consider site conditions and age of samples when testing wood for quality purposes. By keeping these variables as consistent as possible, species specific wood and fibre characteristics may be identifiable.

4.3 Experimental Design and Hypotheses

Stem increment cores were obtained at breast height from samples at each site for fibre property analysis. Cores were analyzed for fibre length and coarseness at different age classes through Fibre Quality Analysis (FQA); microfibril angle, basic density, and cell wall configuration using SilviScan technology. Two 10 mm cores (bark to bark) were taken from each tree sampled.

Thirty samples of pure lodgepole pine, 30 of pure jack pine, and 30 potential hybrid samples were collected from the areas of Prince George, BC, Smoky Lake, AB, and Fort Nelson, BC, respectively. Each site was chosen for its similarity in vegetation and moisture/nutrient regimes. Sites for lodgepole pine and jack pine were chosen as to be similar distances from the hybrid zone.

4.4 Methodology

4.4.1 Fibre Quality Analysis

The Fibre Quality Analyzer (FQA) is an instrument that determines length, shape, average coarseness, and fibre curl and kink of a selected wood sample. It can also determine fibre morphology by combining these wood characteristics, and determine any areas of fibre damage. The FQA utilizes hydrodynamic flow to orient fibres for measurement (Robertson, et.al., 1998).

Using one of the increment cores obtained from each sample tree, wood age 20-40, 40-60, and 60-80 was prepared for analysis.

Sample Preparation and Protocol

Cores were cooked in deionized water at 120°C for 4 hours, drained, and macerated in a solution of 50% acetic acid and 50% hydrogen peroxide at 70°C for 48 hours. After this period, they were rinsed, filtered, and washed into pulp. Pulp was left to air dry for at least 24 hours, and then select samples were oven dried at ~95°C for another 24 hour period to obtain an average moisture content for the pulp samples. Using the average moisture content, oven dry weights were calculated for all samples.

To prepare the samples for input into the FQA, 30 mg of each sample was weighed out and placed into small vials with deionized water. Samples were allowed to sit and reabsorb water until saturated. Each sample was then broken up into single fibres by vigorous mixing and further dilution into 4000 g of water. Concentration of fibre weight (mg) over water weight (g) was then calculated. Based on the concentration calculated, a desired sample weight (water and fibres) was established using a chart provided. The chart displays sample weights for each possible concentration calculated based on a target fibre frequency of 10-20 fibres/second when measured in the FQA, and a sample mass of 1.6 mg for the type of softwoods being analyzed.

After this is complete the sample was then loaded into the FQA for measurement. Output from the FQA analysis included: a fibre distribution table, fibre frequencies, average length weighted fibre length, and average coarseness (mg/m).

4.4.2 SilviScan

SilviScan technology is designed for fast, non-destructive testing of wood. The instrument uses optical microscopy, x-ray diffractometry, x-ray densitometry, image analysis, applied mathematics and analysis of large data sets, to output wood and fibre properties. SilviScan can be used to determine variation in wood chemistry and anatomy for more effective and efficient processing (Paprican press release, 2004).

Using the remaining whole core sampled from select trees, wood was non-destructively analyzed by SilviScan. Fifteen samples displaying average wood properties for each site were selected for this analysis.

Sample Preparation

Cores were selected based on site, in order to get equal representation from all samples locations, and samples were also selected for average representation of fibre traits or demonstration of fibre trait range. Five cores of each species (jack pine, lodgepole pine, and hybrid) were chosen for SilviScan.

Cores were debarked and labeled. Samples were submerged in Ethanol for 96 hours and then air-dried for 24 hours. Cores were shipped to CSIRO in Australia for further preparation and analysis. Further preparation involved cutting the cores into 2 mm x 7 mm strips to form samples that are compatible for use in the x-ray diffractometer, x-ray densitometer, and image analysis systems.

4.4.3 Electron Microscopy

Scanning electron microscopy (SEM) involves scanning an electron beam across a specimen to produce a digital image of that specimen, in this case wood samples. Electrons

are collected from the beam's interaction with the sample, X and Y position data is produced, and contrast and brightness information is used to create the image (Goldstein, 1992).

Sample Preparation

Two samples of each species (jack pine, lodgepole pine and hybrids) were sanded to smooth surfaces for imaging. A razor blade was used to slice a straight cross section surface from each core. Samples were soaked in Ethanol to remove water and air dried before imaging.

4.5 Results

4.5.1 Solid Wood Analysis

Table 11: Average density and moisture content for each sample group.

	Jack Pine	Lodgepole Pine	Hybrid Pine
Avg. calculated density (g/cm3)	0.41	0.43	0.4
Standard Errors for Density	0.006897	0.008577577	0.00447093
Avg. moisture content (% of OD)	73.58	60.17	55.25
Standard Errors for MC	2.838508	2.378054	2.571498
Site index	15	16.1	12
Number of samples	30	30	30

Table 11 shows the average density, average moisture content and site index values for the three species groups. On average densities are comparable, lodgepole pine seems to be slightly higher than the other species. Jack pine has the highest average moisture content, and hybrids have the lowest. Site indices for the sampling areas are highest for lodgepole pine and lowest for hybrid pine.

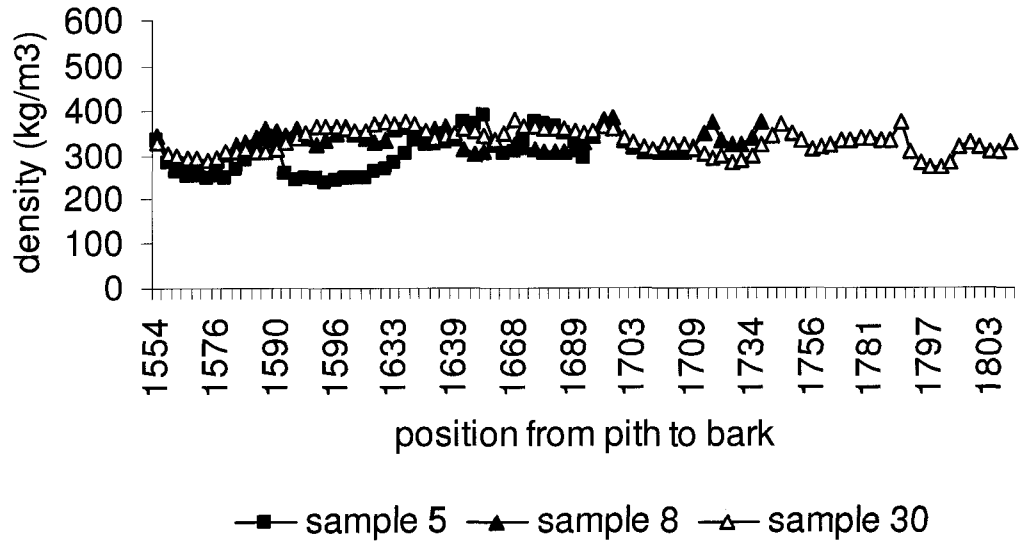


Figure 27a: Jack pine mature wood, earlywood density profile moving from inner wood to bark.

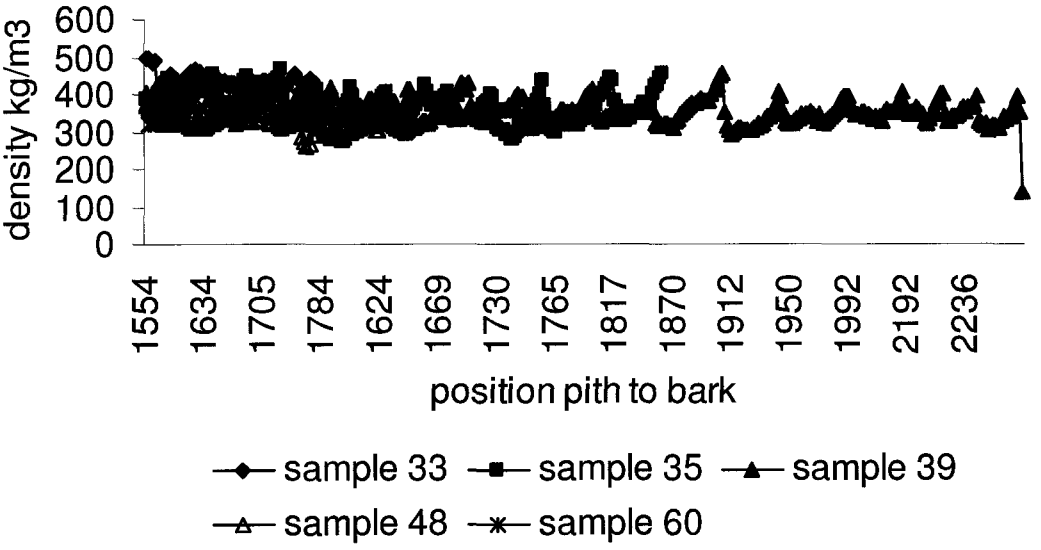


Figure 27b: Lodgepole pine mature wood, earlywood density profile moving from inner wood to bark.

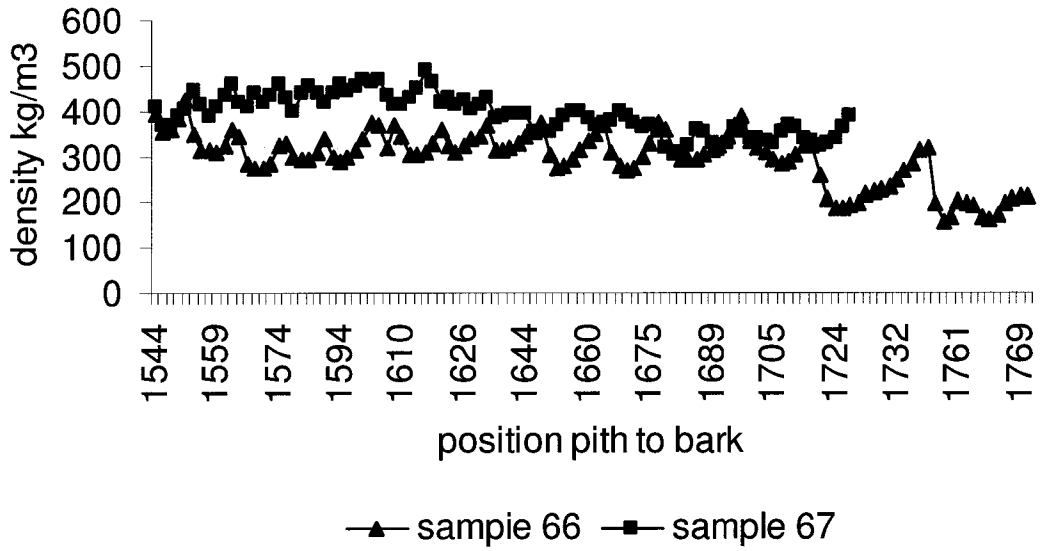


Figure 27c: Hybrid pine mature wood, earlywood density profile moving from inner wood to bark.

Figure 27a, b, and c show that lodgepole pine has the highest density of the three species groups, when considering mature and earlywood distributions. Mature wood and earlywood were separated from the core for consideration because variation exists within the wood, and considering the whole core at once can give skewed averages; juvenile wood and earlywood/latewood ratios have large influences on the overall density of the core. This creates profiles which can be directly compared to each other, irrespective of earlywood/latewood ratio contribution or amount of juvenile wood. Mature wood was considered to be wood over the age of 60 to ensure no juvenile wood component, and earlywood was considered to be wood with a density less than 400 kg/m^3; this density was chosen as the division between the two wood types based on the wood density curves obtained from the samples via SilviScan.

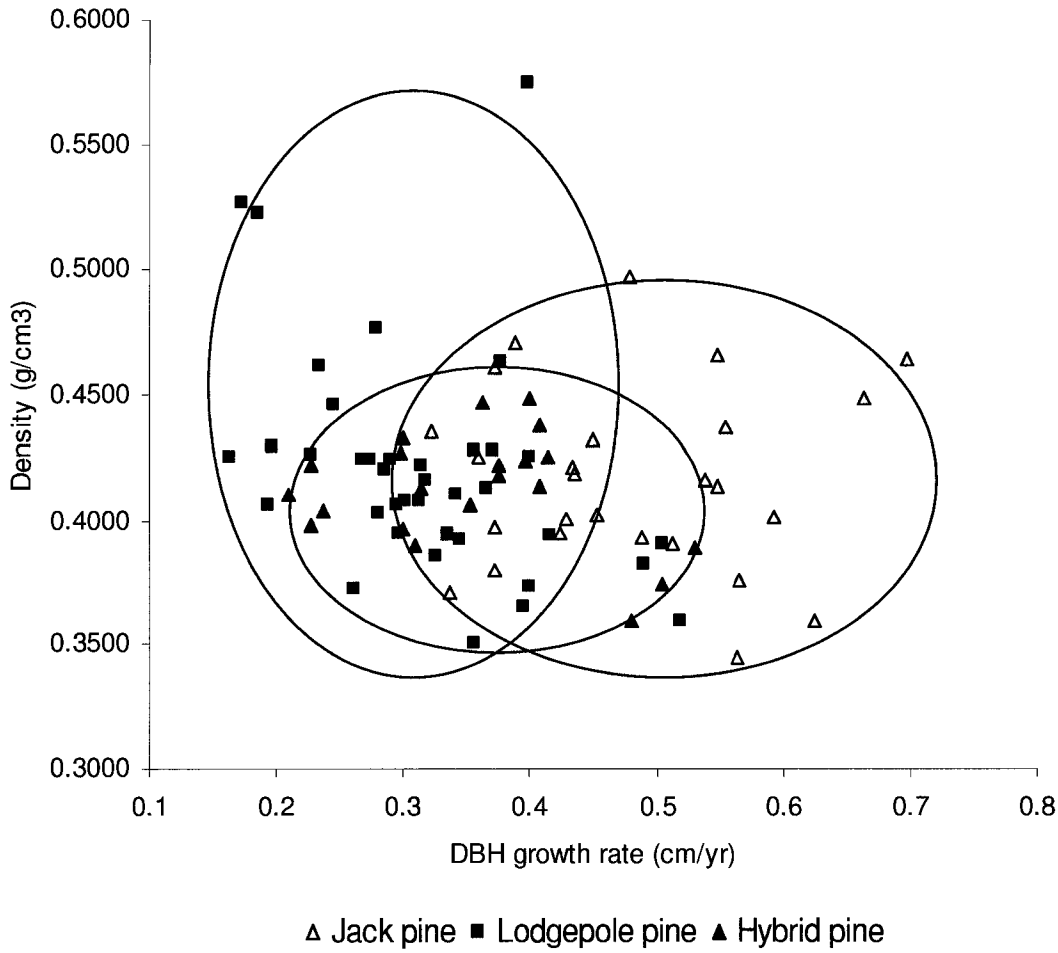


Figure 28a: Calculated density and DBH growth rate based on ring count age, measured DBH, measured volume, and measured weight of cores.

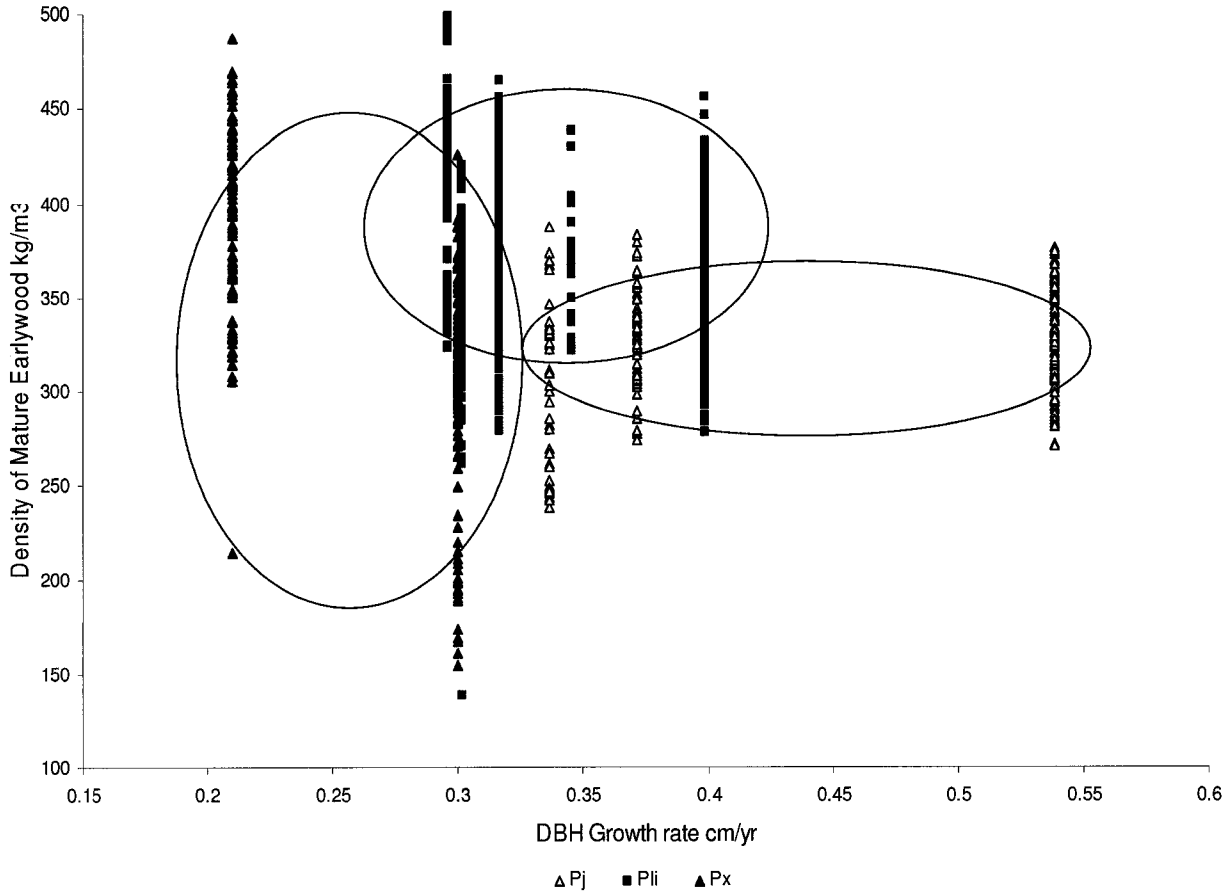


Figure 28b: Density of samples measured by SilviScan that were over 60 years of age plotted against DBH growth rate. Only mature (wood over 60 years of age) earlywood measurements are displayed.

Density was calculated in two ways for the samples collected. All cores collected were measured for volume and weight, which yielded a "calculated" density value. Selected samples that were sent for measurement via SilviScan also returned with a density value, and were used as a comparison to the calculated values obtained. Figure 28a shows the calculated densities, and Figure 28b shows the SilviScan densities of sample groups as they may be related to growth rate. Lodgepole pine and hybrid pine have similar rates of growth according to these figures, but the hybrid sample densities are lower. Jack pine samples have faster growth rates than both lodgepole pine and hybrid samples, but a lower density than lodgepole pine samples. The density values obtained from each method are similar.

Table 12: Pairwise Comparisons of Calculated density and moisture content in all 5 sample groups. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
MC % (OD-GR)	1	2	12.994(*)	3.754	.001	5.525	20.462
		3	22.535(*)	4.093	.000	14.392	30.677
		4	-2.959	7.347	.688	-17.575	11.657
		5	8.697	5.105	.092	-1.458	18.852
	2	1	-12.994(*)	3.754	.001	-20.462	-5.525
		3	9.541(*)	3.994	.019	1.595	17.487
		4	-15.953(*)	7.293	.032	-30.460	-1.445
		5	-4.297	5.026	.395	-14.296	5.702
	3	1	-22.535(*)	4.093	.000	-30.677	-14.392
		2	-9.541(*)	3.994	.019	-17.487	-1.595
		4	-25.493(*)	7.473	.001	-40.359	-10.628
		5	-13.838(*)	5.284	.011	-24.349	-3.326
	4	1	2.959	7.347	.688	-11.657	17.575
		2	15.953(*)	7.293	.032	1.445	30.460
		3	25.493(*)	7.473	.001	10.628	40.359
		5	11.656	8.072	.153	-4.401	27.713
	5	1	-8.697	5.105	.092	-18.852	1.458
		2	4.297	5.026	.395	-5.702	14.296
		3	13.838(*)	5.284	.011	3.326	24.349
		4	-11.656	8.072	.153	-27.713	4.401
Density (g/cm3)	1	2	-.016	.010	.116	-.035	.004
		3	.004	.011	.696	-.017	.026
		4	.021	.019	.285	-.018	.059
		5	.030(*)	.013	.028	.003	.057
	2	1	.016	.010	.116	-.004	.035
		3	.020	.011	.062	-.001	.041
		4	.037	.019	.060	-.002	.075
		5	.046(*)	.013	.001	.019	.072
	3	1	-.004	.011	.696	-.026	.017
		2	-.020	.011	.062	-.041	.001
		4	.017	.020	.401	-.023	.056
		5	.026	.014	.066	-.002	.054
	4	1	-.021	.019	.285	-.059	.018
		2	-.037	.019	.060	-.075	.002
		3	-.017	.020	.401	-.056	.023
		5	.009	.021	.663	-.033	.052

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
	5	1	-.030(*)	.013	.028	-.057	-.003
		2	-.046(*)	.013	.001	-.072	-.019
		3	-.026	.014	.066	-.054	.002
		4	-.009	.021	.663	-.052	.033

Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Cluster Analysis:

Table 13a: Final Cluster Centers for moisture content and density

	Cluster		
	1	2	3
MC % (OD-GR)	67.57130	48.88333	91.63527
Density (g/cm3)	.41507	.42588	.39124

Table 13b: Distances between Final Cluster Centers

Cluster	1	2	3
1		18.688	24.064
2	18.688		42.752
3	24.064	42.752	

Table 13c: Number of Cases in each Cluster

Cluster	1	38.000
	2	37.000
	3	12.000
Valid		87.000
Missing		3.000

Jack pine samples primarily fall into cluster 1, with a small portion of samples in clusters 2 and 3. Approximately half of the lodgepole pine samples fall into cluster 1 as well, and the other half fall into cluster 2 with the exception of samples 44 and 57 that fall into cluster 3. The majority of hybrid samples fall into cluster 2, a portion of the samples fall into

cluster 1. One hybrid sample, 85, falls into cluster 3. Further statistical output is displayed in Appendix 3.

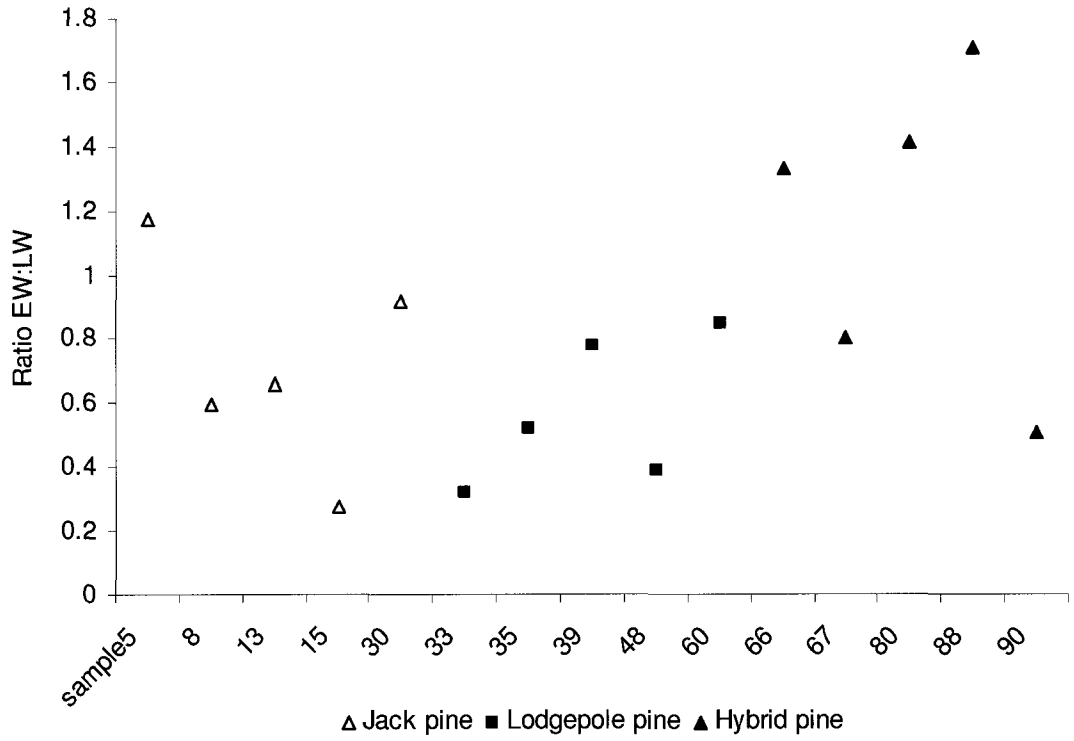


Figure 29: Ratios of earlywood to latewood in each sample. Both Juvenile wood and mature wood were used for the ratio.

SilviScan average EW:LW ratio values are displayed in Figure 29. In general, lodgepole pine and jack pine both have lower EW:LW ratios than hybrid pine, meaning that latewood and earlywood ratios are closer to 1. There is almost 2 times the amount of earlywood to latewood in hybrid samples. This is probably due to site variation and climate.

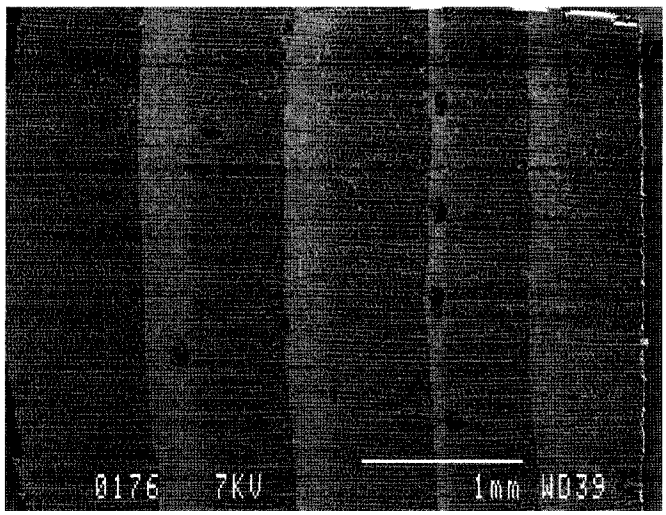


Figure 30a: Earlywood - latewood transition as shown in potential hybrid pine sample #64.

Note the variability in latewood thickness depending on growth influences of site or growing season in Figure 30a.

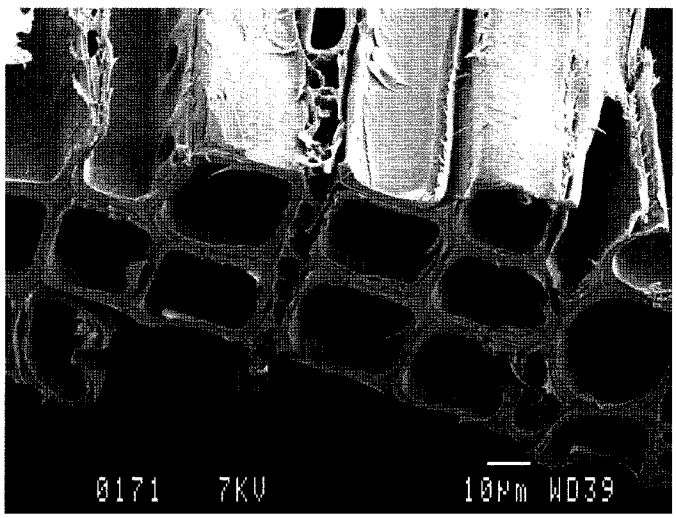


Figure 30b: Jack pine sample cross section with longitudinal view of trachieds.

An example of cell wall thickness and cell dimensions can be easily viewed in Figure 30b.

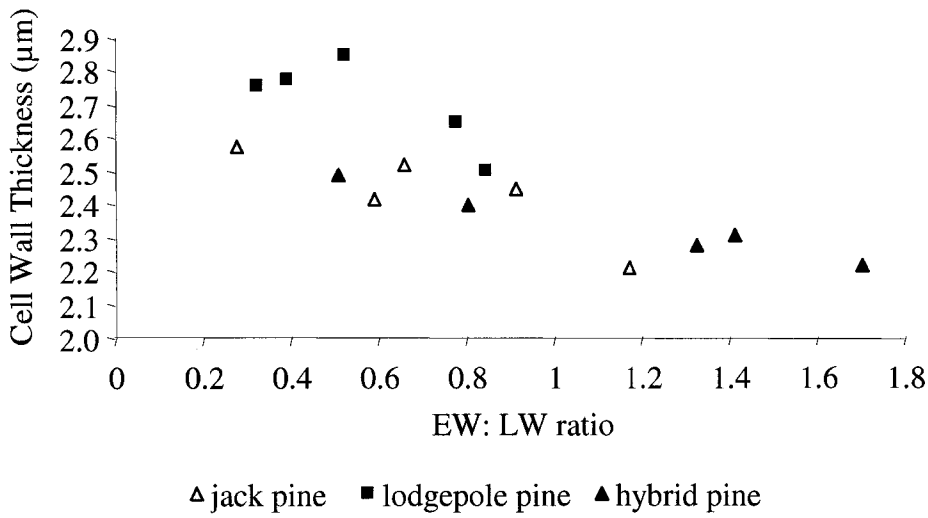


Figure 31a: The relationship between earlywood/latewood contributions and cell wall thickness.

Cell wall thickness and earlywood : latewood ratios have a linear relationship. In Figure 31a, as the percentage of earlywood increases in the sample, the average cell wall thickness decreases. Lodgepole pine samples have the lowest percentage of earlywood, and the thickest average overall cell wall size. Jack pine samples have variable ratios and wall thicknesses, while hybrid samples have samples with thinner walls and more earlywood. Site conditions, specifically latitude and length of growing season are correlated to growth rate and therefore cell wall thickness and EW:LW ratios.

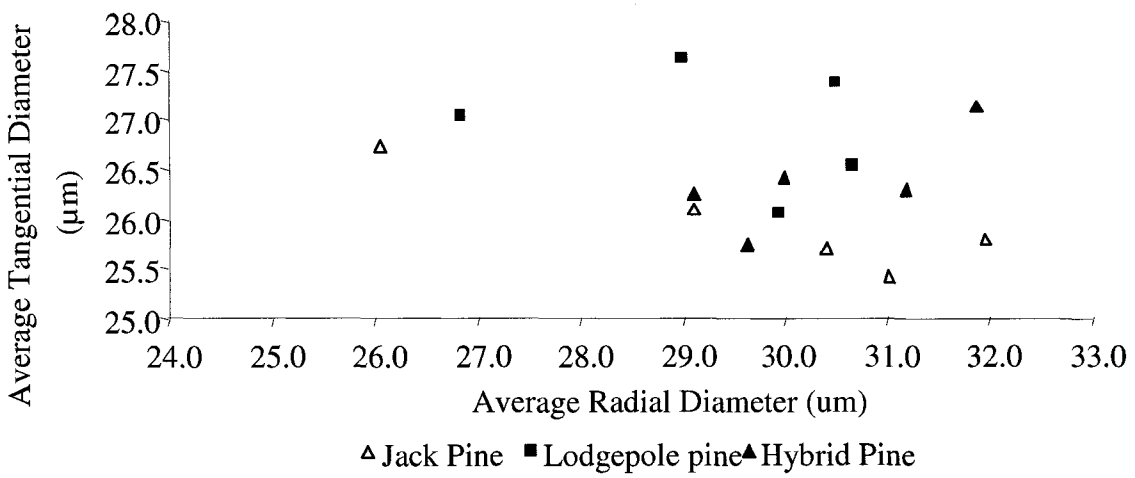


Figure 31b: Average radial and tangential diameter measurements for samples measured by SilviScan.

Figure 31b shows the average dimensions of the cell walls for 5 samples of each species group. Jack pine samples have a larger radial diameter but smaller tangential diameters. Lodgepole pine shows that samples on average have larger tangential diameters than other samples, but smaller radial diameters. Hybrid samples show average diameters scattered in between those of the pure species.

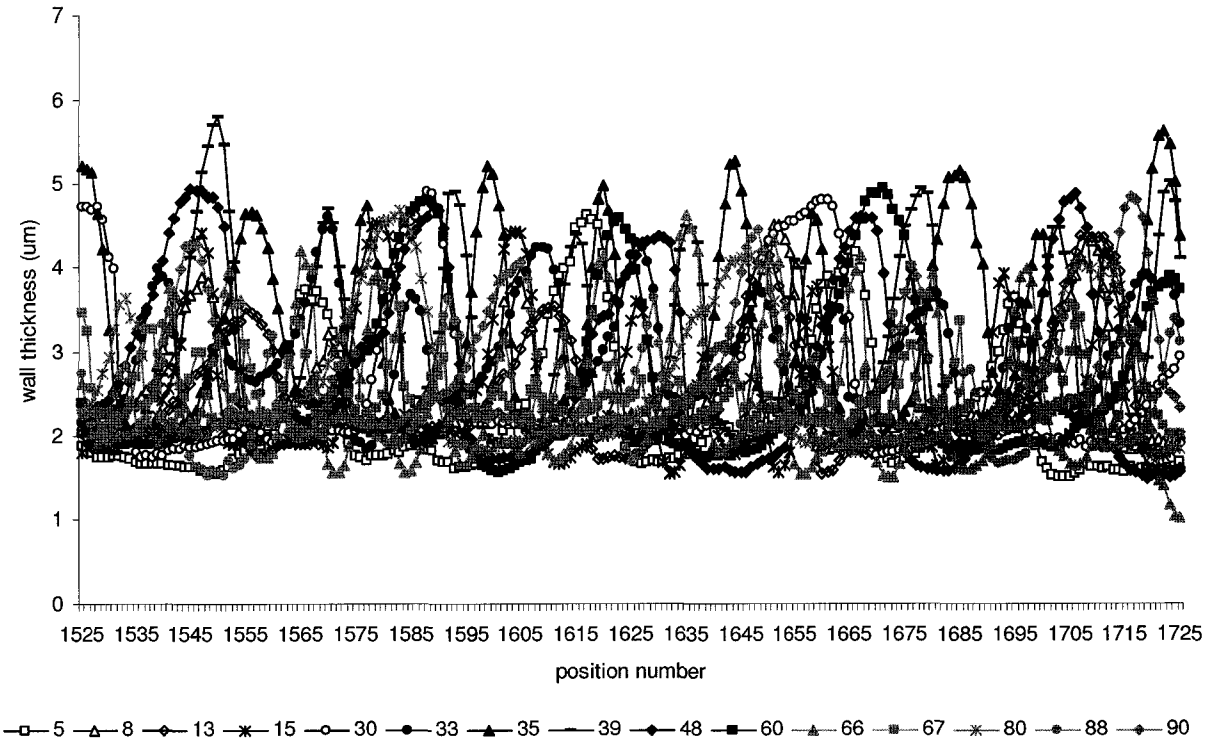


Figure 32: Wall thickness profile for mature wood moving from inner wood to bark. Samples 5-30 are jack pine, 33-60 are lodgepole pine, and 66-90 are hybrid pine.

Figure 32 shows a profile of cell wall thickness for each species. Jack pine and lodgepole pine samples display a greater variation in wall thickness based on this profile, as compared to hybrid samples. It seems that cell walls of mature wood in hybrids may be more consistently thickened than those of pure species.

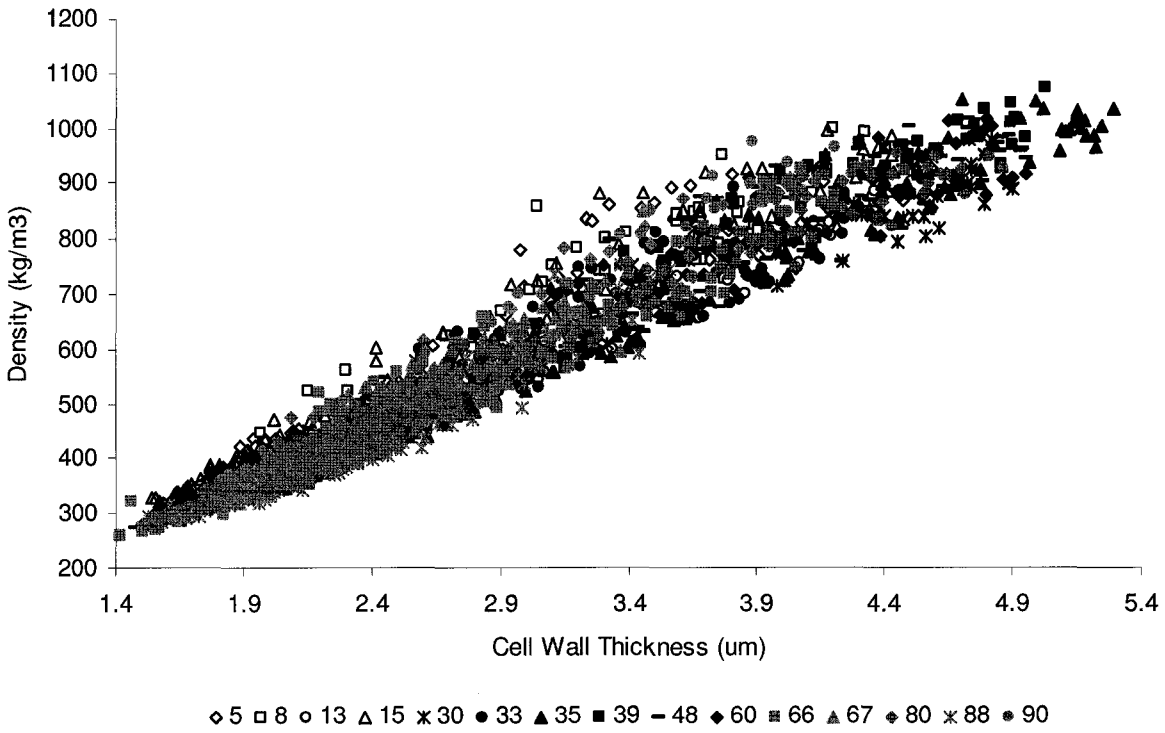


Figure 33: Density as a function of cell wall thickness. Samples 5, 8, 13, 15, and 30 = jack pine; 33, 35, 39, 48, 60 = lodgepole pine; 66, 67, 80, 88, 90 = hybrids

Little difference exists between overall wall thickness profiles with respect to earlywood and latewood densities. Figure 33 shows all cell wall thickness profiles as they are related to density, for comparison between species. The only differences that seem to exist between species are the concentration of samples that are less-dense with thinner walls, as compared to the concentration of samples that are denser with thicker walls. Lodgepole pine has more cells with denser, thicker walls than the other two species. It should be noted that there is a very strong linear relationship between density and cell wall thickness. As cell wall thickness increases, the density of the sample increases.

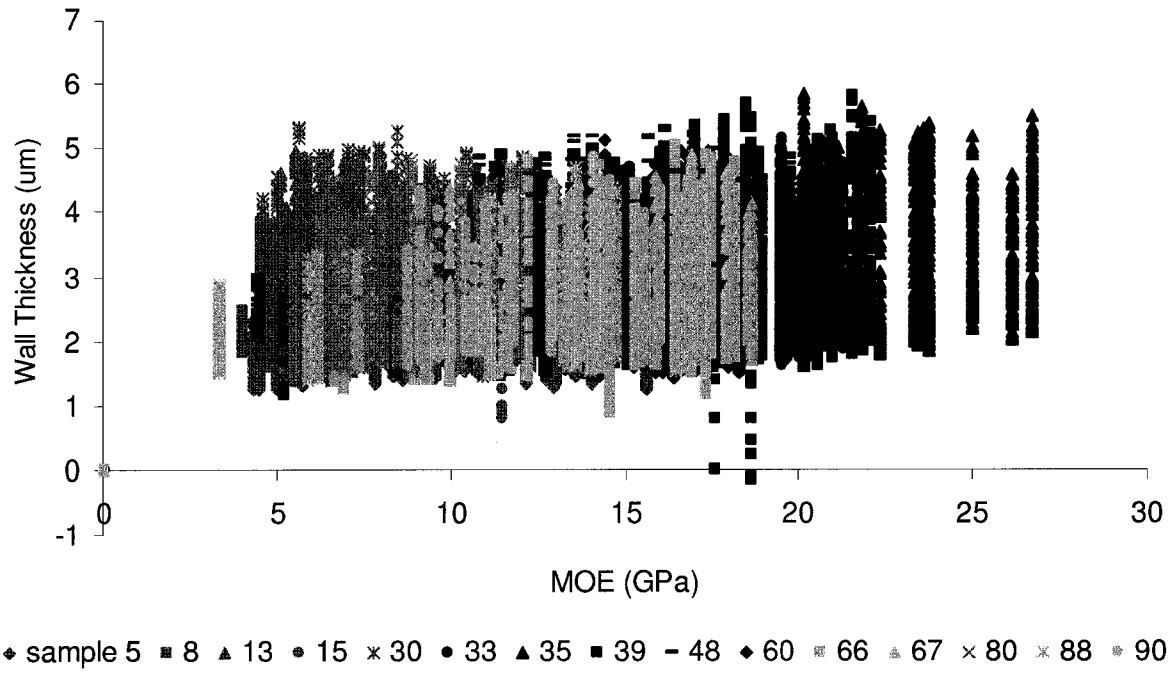


Figure 34: Wall thickness as a function of Modulus of elasticity. All wood (juvenile and mature) included in profile. Samples 5, 8, 13, 15, 30 = jack pine; samples 33, 35, 39, 48, 60 = lodgepole pine; samples 66, 67, 80, 88, 90 = hybrid pine

Modulus of elasticity is measured in Figure 34 as a function of wall thickness. Lodgepole pine samples have a greater MOE than jack pine, and hybrid pines have a moderate MOE, in between that of the pure species. However, there does not appear to be any variation due to the wall thickness, therefore it can be assumed that there is no prominent relationship between MOE and cell wall thickness for these samples.

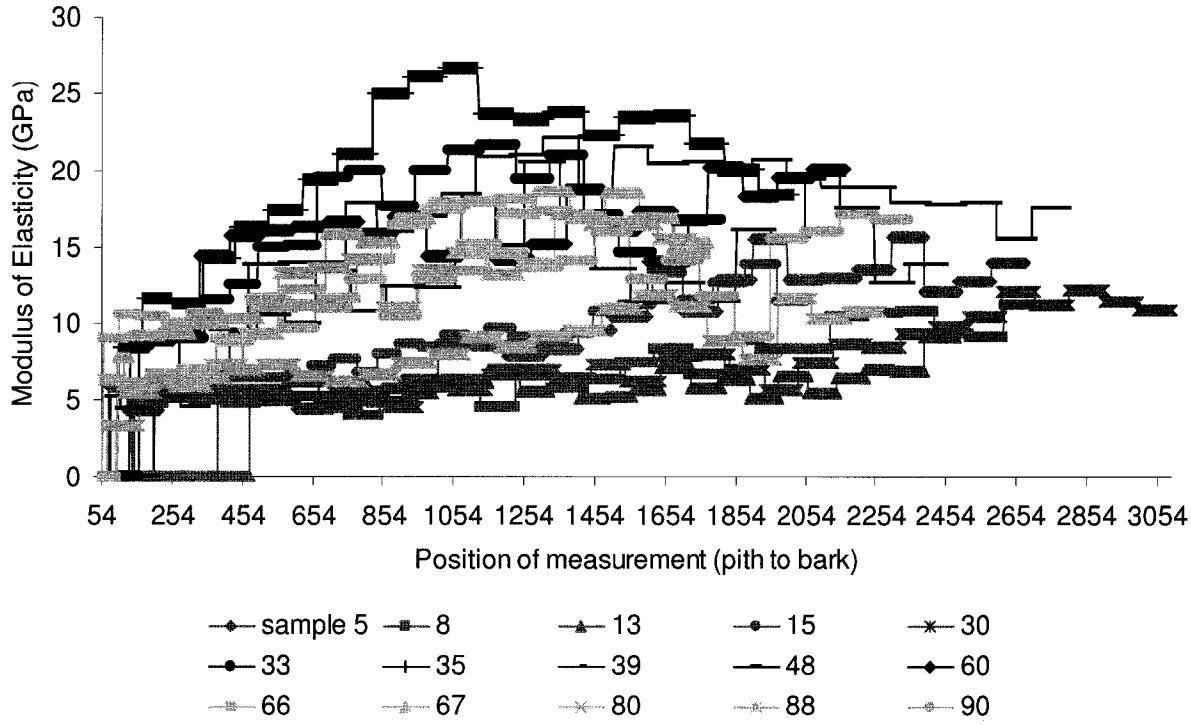


Figure 35: Modulus of Elasticity (MOE) profile from pith to bark for all samples. Mature wood component begins after approximately position 1500.

Figure 35 shows the profile of MOE moving from pith to bark. It is visible that there are some main differences between each species as MOE changes over the growth of the trees. Jack pine samples have a slow but general increase in MOE as the measurements move from pith to bark. Lodgepole pine samples start at a low MOE in the juvenile wood, then increase to a high MOE just before maturity (60 years or ~position 1500), and slowly decrease or level off in MOE as age increases past maturity. Hybrid pine seems to be between these two patterns, where some samples have an increase around the same period as lodgepole pine but some maintain a steady increase like jack pine samples. In both cases the MOE values for hybrid are intermediate of the pure species values.

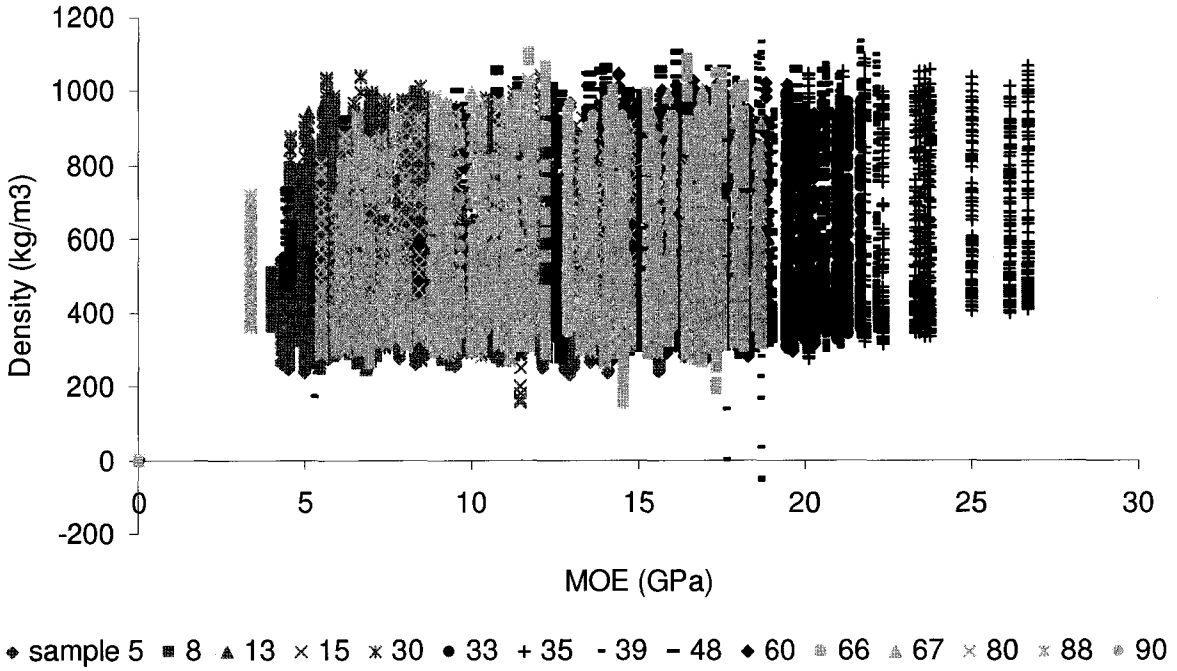


Figure 36: Density as a function of MOE for all samples. All wood present in profile (mature and juvenile).

Figure 36 shows density as a function of MOE. There is no discernable trend between MOE and density according to this figure, however, it can be noted, that lodgepole pine occupies the upper range in MOE value, while jack pine occupies the lower range of MOE value. Hybrid samples measured MOE values fall in between the pure species measurements.

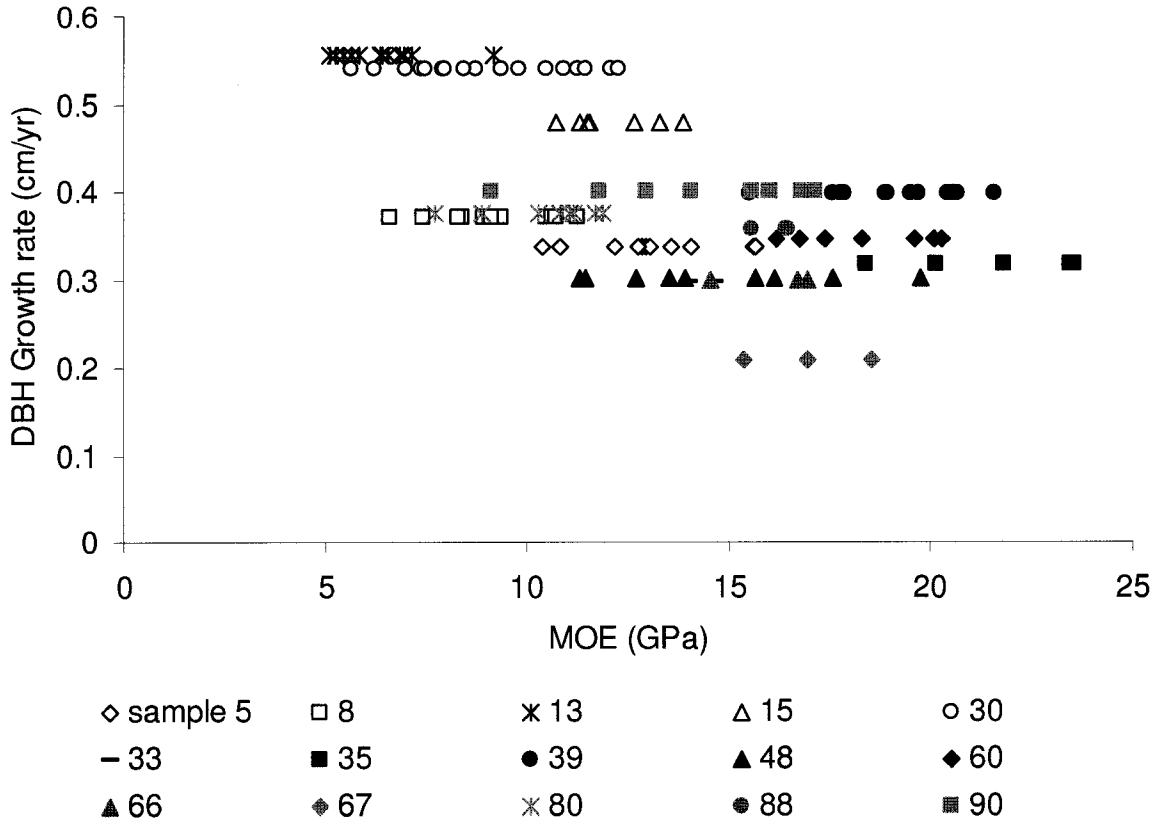


Figure 37: MOE and growth rate (DBH/age) for mature wood MOE measurements.

Figure 37 shows that there is a possible relationship between MOE and growth rate of samples. The samples with slower growth rates seem to have higher MOE values. The jack pine samples have faster rates of growth, and have lower MOE values. Lodgepole pine samples were grown more slowly, and are shown to have greater MOE values on average (Figure 37). Hybrid pine samples also have lower rates of growth, and show intermediate MOE values. This suggests that at the same growth rate, MOE is higher in lodgepole pine than in hybrids. Only mature wood MOE measurements were used in this figure, so juvenile wood should not have influenced the results, however, there are differences in site conditions which may have played a role in the variability of MOE.

Table 14: Pairwise Comparisons for wood characteristics. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
MOE(ave)	1	2	-8.655(*)	1.415	.000	-11.769	-5.541
		3	-4.182(*)	1.634	.027	-7.778	-.587
		5	-4.502(*)	1.872	.035	-8.621	-.383
	2	1	8.655(*)	1.415	.000	5.541	11.769
		3	4.473(*)	1.634	.019	.877	8.069
		5	4.153(*)	1.872	.048	.034	8.273
	3	1	4.182(*)	1.634	.027	.587	7.778
		2	-4.473(*)	1.634	.019	-8.069	-.877
		5	-.320	2.042	.878	-4.814	4.175
	5	1	4.502(*)	1.872	.035	.383	8.621
		2	-4.153(*)	1.872	.048	-8.273	-.034
		3	.320	2.042	.878	-4.175	4.814
ew/lw ratio	1	2	.152	.242	.543	-.380	.684
		3	-.456	.279	.130	-1.071	.158
		5	-.384	.320	.255	-1.088	.320
	2	1	-.152	.242	.543	-.684	.380
		3	-.608	.279	.052	-1.222	.006
		5	-.536	.320	.122	-1.239	.168
	3	1	.456	.279	.130	-.158	1.071
		2	.608	.279	.052	-.006	1.222
		5	.073	.349	.839	-.695	.840
	5	1	.384	.320	.255	-.320	1.088
		2	.536	.320	.122	-.168	1.239
		3	-.073	.349	.839	-.840	.695
Wall(ave)	1	2	-.272(*)	.084	.008	-.457	-.088
		3	.107	.097	.290	-.105	.320
		5	.081	.111	.482	-.163	.324
	2	1	.272(*)	.084	.008	.088	.457
		3	.380(*)	.097	.002	.167	.593
		5	.353(*)	.111	.009	.109	.597
	3	1	-.107	.097	.290	-.320	.105
		2	-.380(*)	.097	.002	-.593	-.167
		5	-.027	.121	.829	-.293	.239
	5	1	-.081	.111	.482	-.324	.163
		2	-.353(*)	.111	.009	-.597	-.109
		3	.027	.121	.829	-.239	.293
mw dens	1	2	-55.308(*)	19.722	.017	-98.715	-11.901
		3	6.650	22.773	.776	-43.472	56.772
		5	17.552	26.089	.515	-39.870	74.974
	2	1	55.308(*)	19.722	.017	11.901	98.715

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
		3	61.958(*)	22.773	.020	11.836	112.080
		5	72.860(*)	26.089	.018	15.438	130.282
	3	1	-6.650	22.773	.776	-56.772	43.472
		2	-61.958(*)	22.773	.020	-112.080	-11.836
		5	10.902	28.466	.709	-51.751	73.554
	5	1	-17.552	26.089	.515	-74.974	39.870
		2	-72.860(*)	26.089	.018	-130.282	-15.438
		3	-10.902	28.466	.709	-73.554	51.751

Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Cluster Analysis:

Table 15a: Final Cluster Centers

	Cluster		
	1	2	3
ew/lw ratio	.81309	.41212	1.12401
mw dens	503.09882	564.84911	454.27874
MOE(ave)	11.00200	16.69203	11.55344
Wall(ave)	2.44608	2.78952	2.34982

Table 15b: Distances between Final Cluster Centers

Cluster	1	2	3
1		62.014	48.824
2	62.014		110.693
3	48.824	110.693	

Table 15c: Number of samples per cluster

Cluster	1	8.000
	2	3.000
	3	4.000
Valid		15.000
Missing		75.000

The cluster analysis revealed that 4 out of 5 jack pine samples fell into cluster 1. Three out of 5 lodgepole pine samples fell into cluster 2 and the other 2 fell into cluster 1. Two out

of 3 hybrid samples fell into cluster 3 and the other 1 fell into cluster 1. The two samples representing species group five, which could be hybrids or lodgepole pine samples, had one sample in cluster 1 and one in cluster 3.

Further statistical output can be found in Appendix 3.

4.5.2 Fibre Analysis:

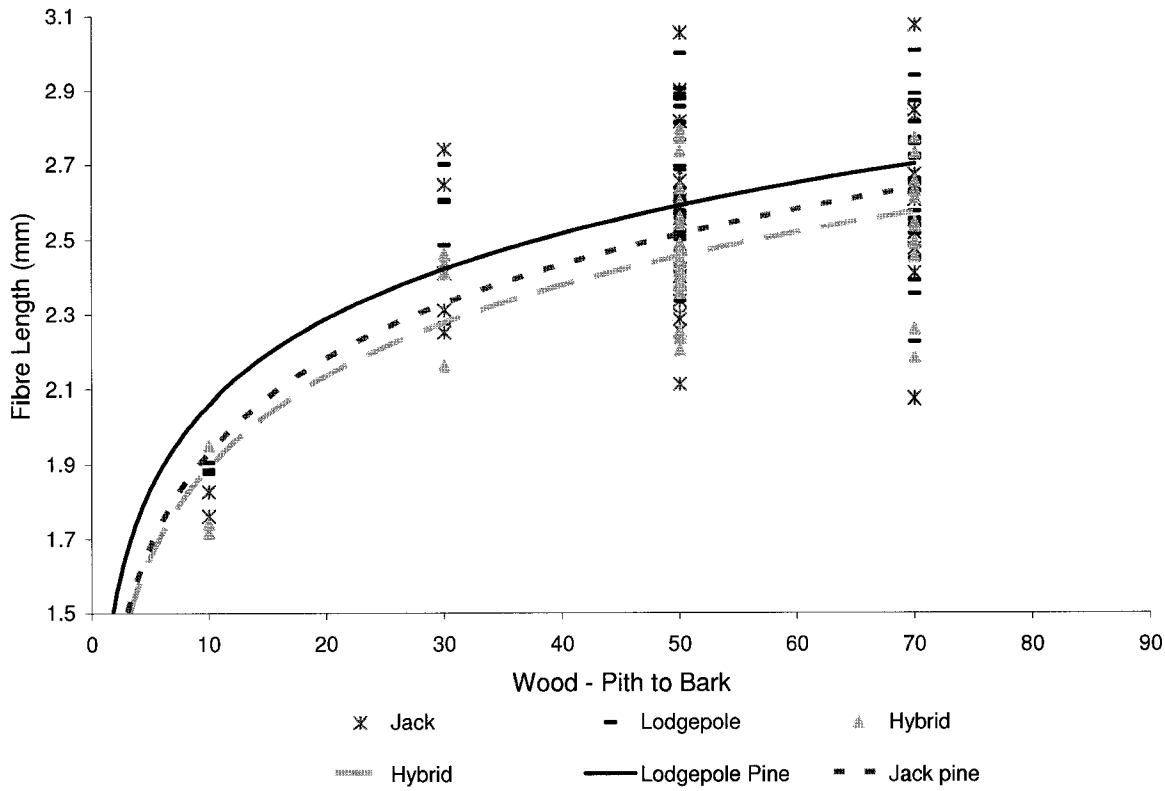


Figure 38: Length weighted fibre length from pith to bark in all species/sample groups with observed trend lines.

Figure 38 shows length weighted fibre length as samples move from 0-20 age class (0-20 rings from the pith) to 60-80 age class (60-80 rings from the pith). Curves for the three species groups are similar, increasing in fibre length in juvenile wood, and forming an asymptote as wood becomes more mature. Throughout the age classes, lodgepole pine has the longest fibres on average, and hybrid fibres are the shortest.

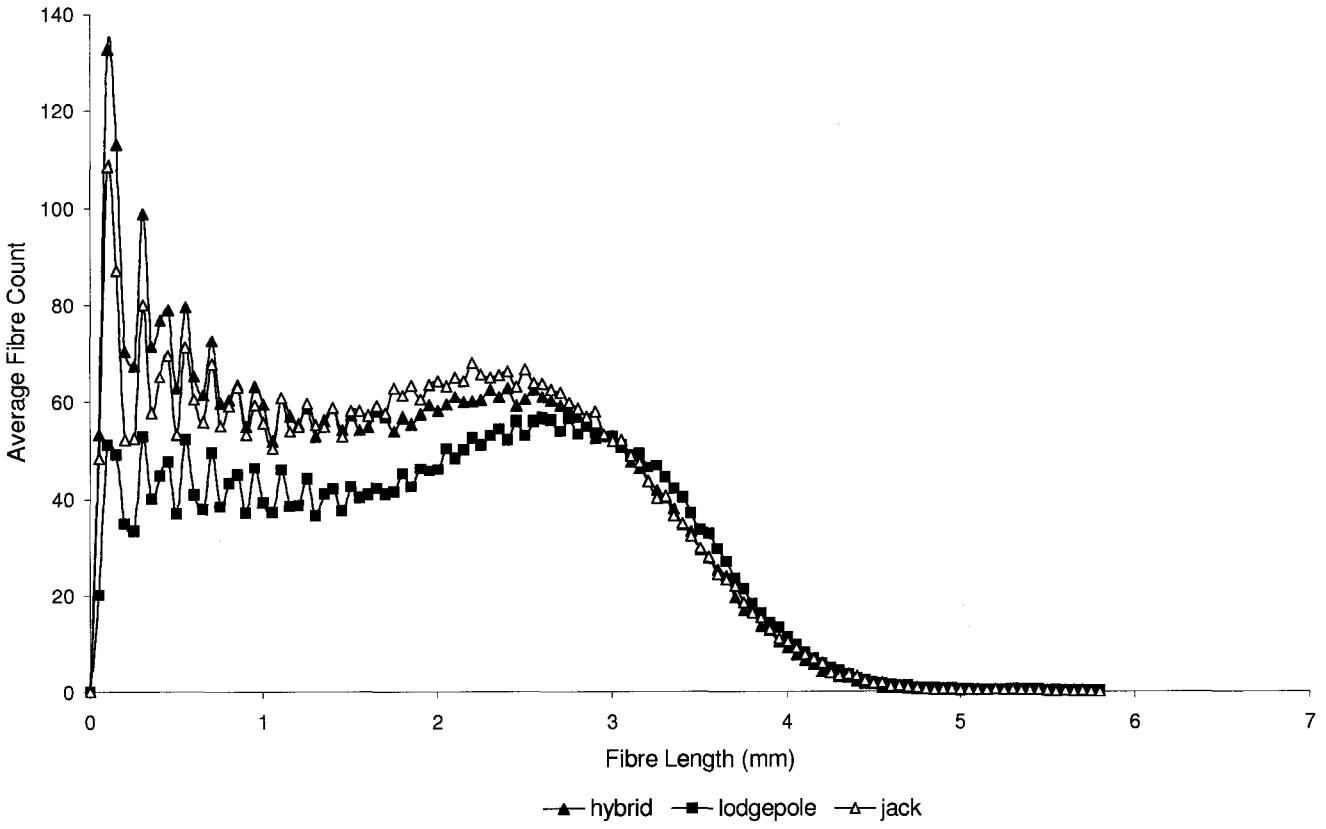


Figure 39: Average number of fibres per fibre length interval for wood rings aged 40-60

Fibre length distributions were calculated from analysis with the FQA. Intervals of fibre lengths were established, starting with very short fibres, shown on the left of Figure 39, and working up to the longest fibres found in the samples on the right. This figure displays the number of fibres found within each one of these length intervals. The longest fibres found were around 6 mm in length. The shortest fibres found were 0.05 mm in length. All three species groups have an increase in number of fibres between 2 and 3 mm in length, after which length the fibres become fewer and fewer. Jack pine samples show more fibres at shorter lengths than lodgepole pine. Hybrid fibres follow a similar curve to jack pine fibres.

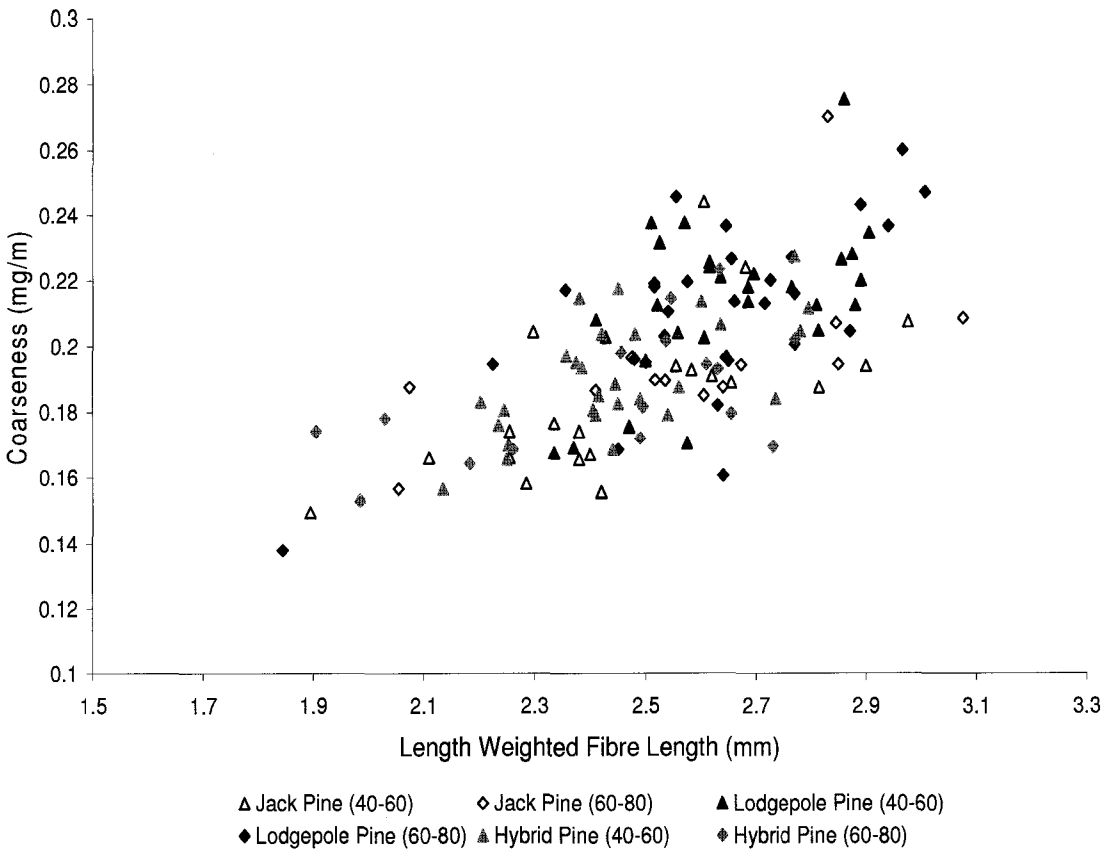


Figure 40: Fibre coarseness as a function of fibre length. Approximate groupings can be considered as outlined by ovals. Measurements by FQA.

Figure 40 shows the relationship between coarseness and length weighted fibre length for all samples in mature wood groups 40-60 years, and 60-80 years. There is definite variability within sample groups, however a general trend is evident in the majority of samples. Jack pine samples are, in general, lower in coarseness and fibre length values than lodgepole pine samples. Hybrid samples seem to be intermediate in both coarseness and fibre length according to this distribution.

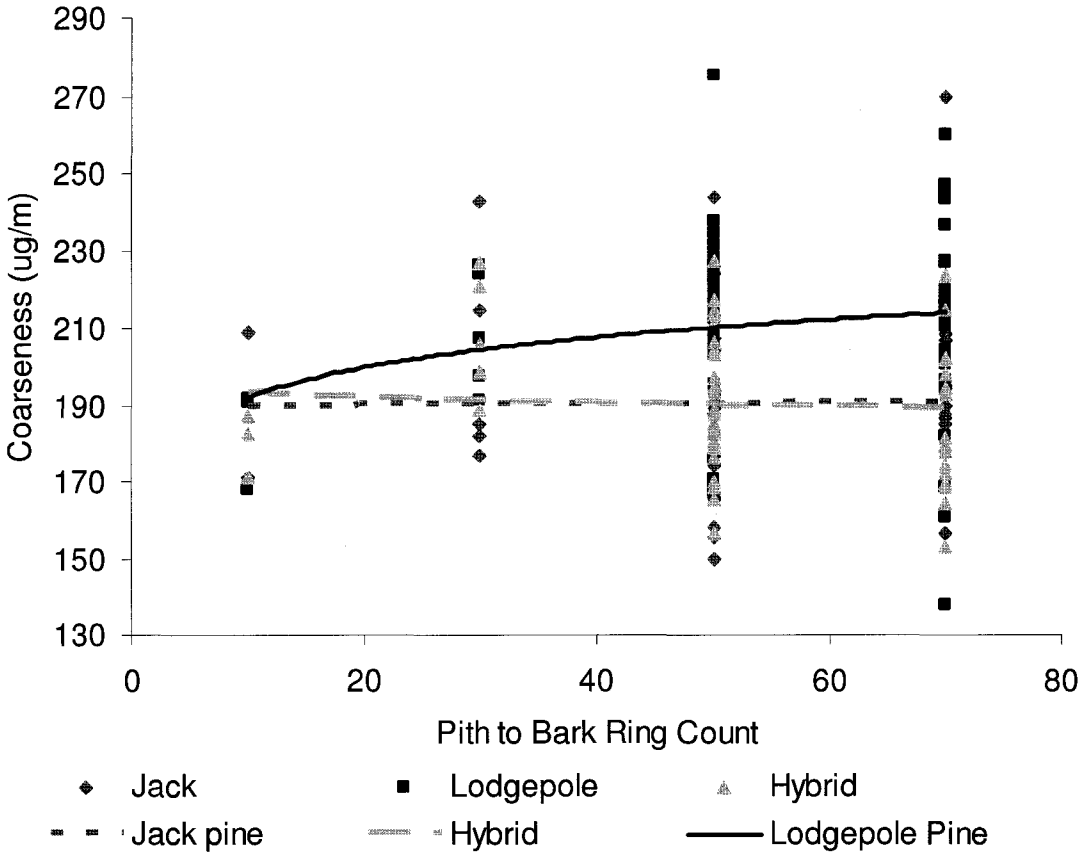


Figure 41: Coarseness profile from FQA measurements moving from pith to bark with observed trend lines.

A coarseness profile moving from pith to bark is displayed in Figure 41. Based on the trend lines established, lodgepole pine has higher coarseness values as the tree matures than those of jack pine or hybrid pine. The coarseness values for jack pine and hybrids remain relatively unchanged moving from pith to bark (juvenile to mature wood) in these samples. By the 60-80 year ring count, the jack pine and hybrid coarseness values seem to be much lower on average than the lodgepole pine coarseness values.

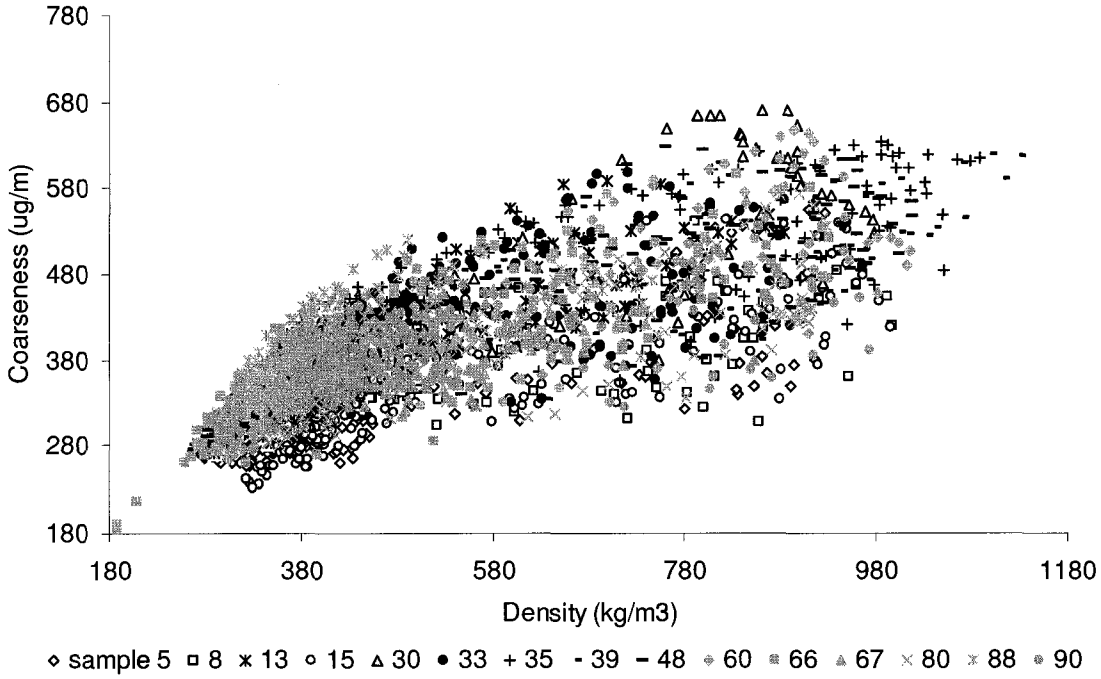


Figure 42: Fibre coarseness as a function of density for mature wood only.

Figure 42 shows that with increasing fibre coarseness, density increases. All samples have a concentration of values at lower coarseness and density, while distributions become increasingly variable as coarseness and density get higher. Jack pine has the most variability within samples, while lodgepole pine has the least variability. At lower values of coarseness and density, hybrid pine samples follow a lodgepole pine pattern, and jack pine samples are slightly different. Lodgepole pine samples have the highest density and coarseness values among the three species groups.

Table 16: Pairwise Comparisons of fibre length and coarseness for wood age 40-80. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
Fibre length (40-60)	1	2	-.014	.072	.848	-.160	.132
		3	.099	.082	.231	-.065	.264
		4	.339	.204	.103	-.072	.750
	2	1	.014	.072	.848	-.132	.160
		3	.113	.066	.093	-.020	.246
		4	.353	.198	.082	-.046	.752
	3	1	-.099	.082	.231	-.264	.065
		2	-.113	.066	.093	-.246	.020
		4	.240	.202	.241	-.167	.646
	4	1	-.339	.204	.103	-.750	.072
		2	-.353	.198	.082	-.752	.046
		3	-.240	.202	.241	-.646	.167
Fibre length (60-80)	1	2	.004	.074	.957	-.145	.153
		3	.114	.084	.181	-.055	.282
		4	.047	.209	.825	-.373	.466
	2	1	-.004	.074	.957	-.153	.145
		3	.110	.068	.111	-.026	.246
		4	.043	.203	.835	-.365	.450
	3	1	-.114	.084	.181	-.282	.055
		2	-.110	.068	.111	-.246	.026
		4	-.067	.206	.746	-.483	.348
	4	1	-.047	.209	.825	-.466	.373
		2	-.043	.203	.835	-.450	.365
		3	.067	.206	.746	-.348	.483
Fibre coarseness (40-60)	1	2	-.017(*)	.007	.021	-.032	-.003
		3	.001	.008	.913	-.016	.018
		4	.036	.021	.087	-.005	.078
	2	1	.017(*)	.007	.021	.003	.032
		3	.018(*)	.007	.008	.005	.032
		4	.054(*)	.020	.010	.013	.094
	3	1	-.001	.008	.913	-.018	.016
		2	-.018(*)	.007	.008	-.032	-.005
		4	.035	.020	.092	-.006	.076
	4	1	-.036	.021	.087	-.078	.005
		2	-.054(*)	.020	.010	-.094	-.013
		3	-.035	.020	.092	-.076	.006

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
Fibre coarseness (60-80)	1	2	-.011	.008	.139	-.027	.004
		3	.013	.009	.131	-.004	.030
		4	.017	.021	.415	-.025	.060
	2	1	.011	.008	.139	-.004	.027
		3	.024(*)	.007	.001	.011	.038
		4	.029	.021	.169	-.013	.070
	3	1	-.013	.009	.131	-.030	.004
		2	-.024(*)	.007	.001	-.038	-.011
		4	.004	.021	.836	-.038	.047
	4	1	-.017	.021	.415	-.060	.025
		2	-.029	.021	.169	-.070	.013
		3	-.004	.021	.836	-.047	.038

Based on estimated marginal means

* The mean difference is significant at the .05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 17: Pairwise Comparisons for fibre length and coarseness of wood age 20-40. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
Fibre length (20-40)	1	2	-.062	.110	.585	-.304	.180
		3	.036	.127	.782	-.244	.316
		5	.161	.146	.293	-.160	.482
	2	1	.062	.110	.585	-.180	.304
		3	.098	.127	.457	-.182	.378
		5	.223	.146	.154	-.098	.544
	3	1	-.036	.127	.782	-.316	.244
		2	-.098	.127	.457	-.378	.182
		5	.125	.159	.448	-.225	.475
	5	1	-.161	.146	.293	-.482	.160
		2	-.223	.146	.154	-.544	.098
		3	-.125	.159	.448	-.475	.225
coarseness (20-40)	1	2	-.009	.012	.478	-.036	.018
		3	.003	.014	.863	-.029	.034
		5	-.024	.016	.178	-.060	.013
	2	1	.009	.012	.478	-.018	.036
		3	.012	.014	.433	-.020	.043
		5	-.014	.016	.397	-.051	.022

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
	3	1	-.003	.014	.863	-.034	.029
		2	-.012	.014	.433	-.043	.020
		5	-.026	.018	.172	-.065	.013
	5	1	.024	.016	.178	-.013	.060
		2	.014	.016	.397	-.022	.051
		3	.026	.018	.172	-.013	.065

Based on estimated marginal means

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustment

Cluster Analysis:

Table 18a: Final Cluster Centers for fibre length and coarseness at 40-60 and 60-80 yrs.

	Cluster		
	1	2	3
Fibre length (40-60)	2.51	2.36	2.82
Fibre length (60-80)	2.59	2.32	2.79
Fibre coarseness (40-60)	.198	.189	.214
Fibre coarseness (60-80)	.20	.19	.22

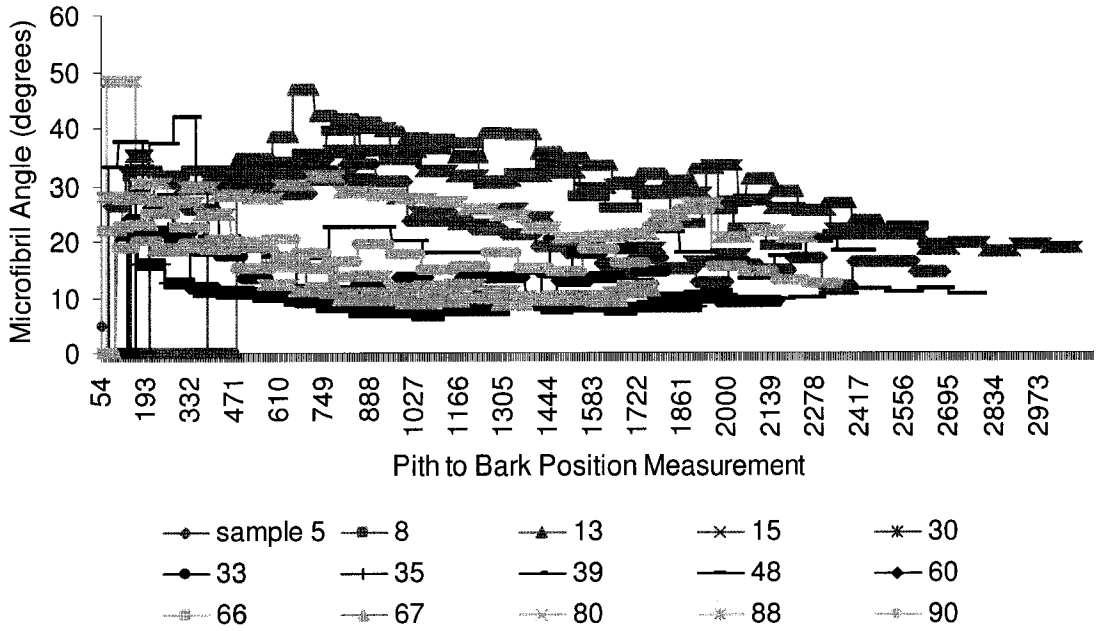
Table 18b: Distances between Final Cluster Centers

Cluster	1	2	3
1		.313	.359
2	.313		.652
3	.359	.652	

Table 18c: Number of Cases in each Cluster

Cluster	1	24.000
	2	8.000
	3	18.000
Valid		50.000
Missing		43.000

A small majority of the jack pine samples fell into cluster 1, and a small majority of the lodgepole pine samples fell into cluster 3. A large majority of the hybrid samples fell into cluster 1. Cluster 2 is composed of a few samples from each species group. Further statistical data can be found in Appendix 3.



43: Microfibril angle profile moving from pith to bark.

Figure 43 displays a profile of the microfibril angle moving from pith to bark in select samples. Jack pine samples (samples 5, 8, 13, 15, 30) show higher microfibril angles than both lodgepole pine (samples 33, 35, 39, 48, 60) and hybrids (samples 66, 67, 80, 88, 90) throughout the entire core of most of the samples. Lodgepole pine has the lowest overall microfibril angle, while hybrid samples contain more variability but parallel lodgepole pine samples over jack pine samples.

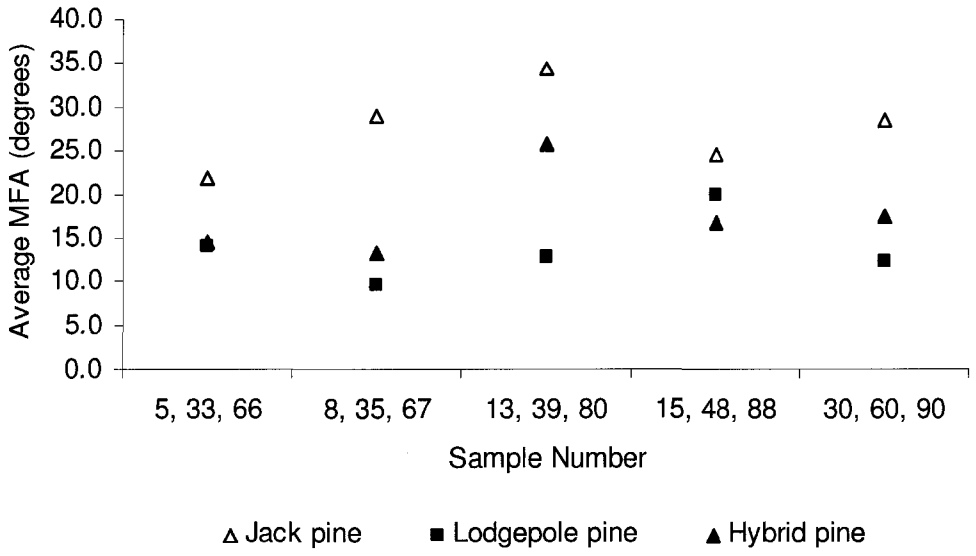


Figure 44: Average microfibril angle for select samples representing sampling areas. Pj – sample 5, 8, 13, 15, 30; Pli – sample 33, 35, 39, 48, 60; Px – sample 66, 67, 80, 88, 90.

Figure 44 shows microfibril angles averaged over the whole core of each sample, for all three species groups. Both juvenile wood and mature wood are included in the average, so no differentiation can be made between angles of the different wood types. However, it can be observed that jack pine has an overall higher microfibril angle than lodgepole or hybrid pine in all the samples measured. This also coordinates with the results gathered from Figure 43, which displayed that jack pine had a higher microfibril angle in mature wood as well as juvenile wood. Some samples may contain more juvenile wood than others, which would make the average microfibril angle higher for samples in Figure 44.

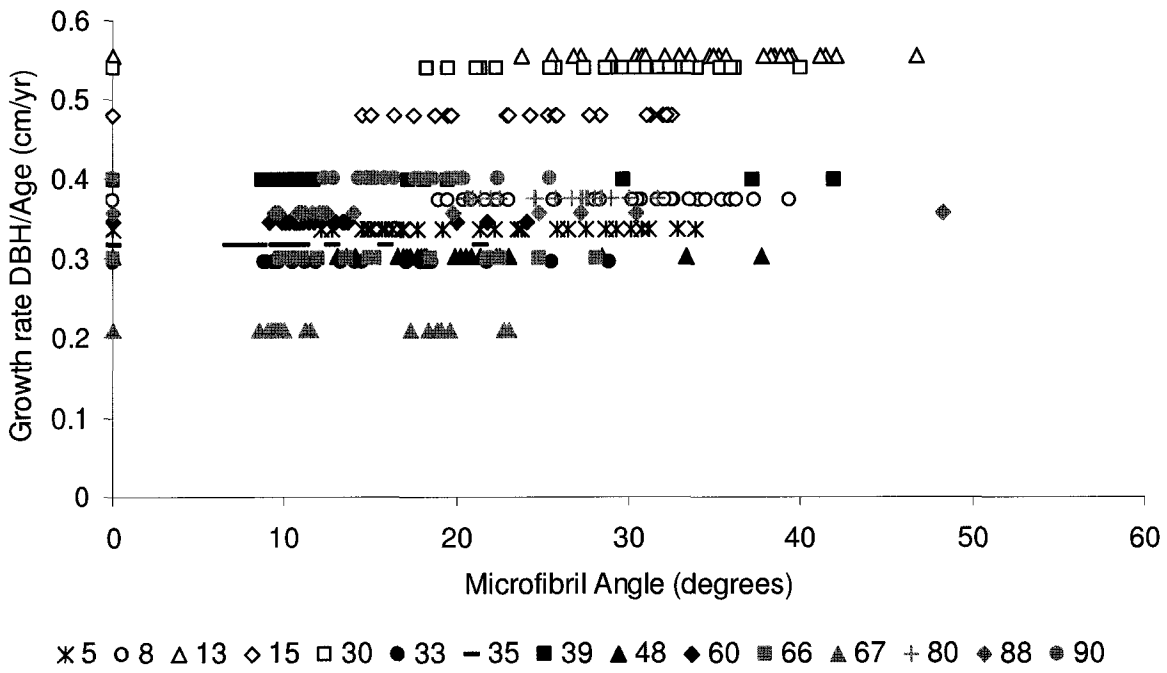


Figure 45: DBH growth rate as a function of microfibril angle. Samples 5, 8, 13, 15, 30 = jack pine; samples 33, 35, 39, 48, 60 = lodgepole pine; samples 66, 67, 80, 88, 90 = hybrid pine.

Figure 45 displays DBH growth rate as a function of MFA. Jack pine samples have the highest growth rate and highest MFA according to this figure, while lodgepole pine has a lower MFA and growth rate. Hybrid pine has the lowest representation of growth rate, and microfibril angles comparable to those of lodgepole pine. This figure suggests that microfibril angle may be influenced by growth rate for these samples.

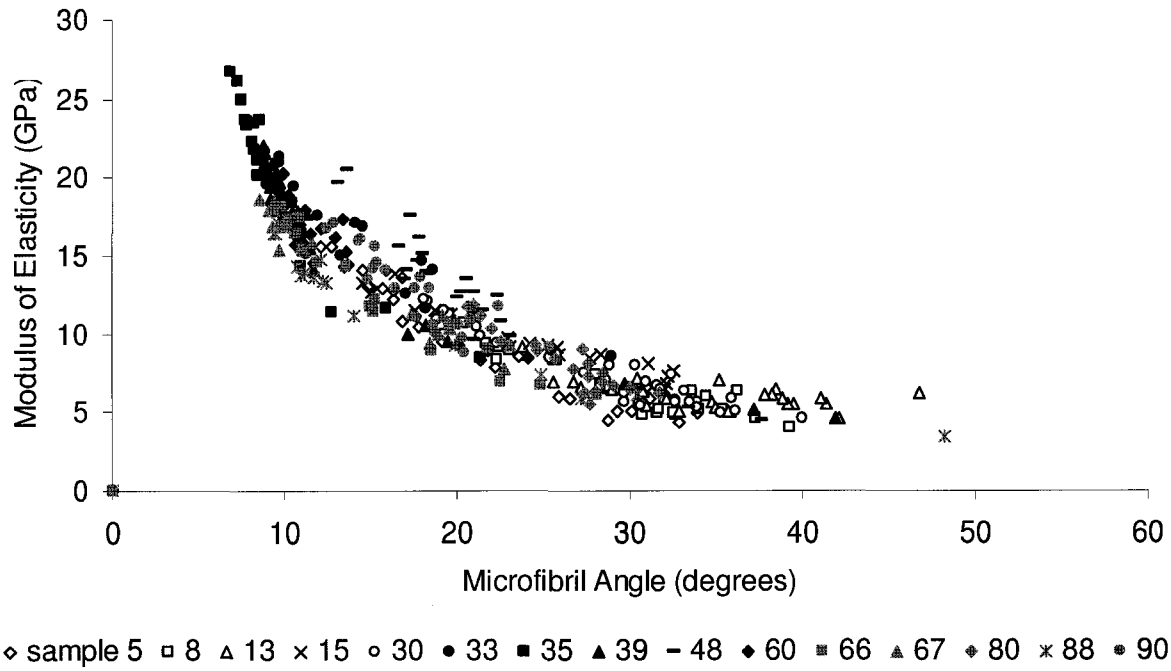


Figure 46: MOE as a function of microfibril angle.

Figure 46 displays microfibril angle and modulus of elasticity. There is a clear relationship between these two properties as can be seen in this figure. As MFA increases, MOE decreases in a log rhythmic shape, forming asymptotes at the x and y axes. Jack pine samples are found at the lower end of the MOE scale, but at the highest MFA values. Lodgepole pine samples have low MFA values (as seen in previous figures) and relatively high MOE values. Hybrid samples are found intermediate of both species, with moderate values for both MFA and MOE.

Table 19: Pairwise Comparisons of fibre traits. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
Crs(ave)	1	2	-36.712(*)	12.089	.011	-63.320	-10.105
		3	15.176	13.959	.300	-15.548	45.900
		5	-9.443	15.992	.567	-44.641	25.756
	2	1	36.712(*)	12.089	.011	10.105	63.320
		3	51.888(*)	13.959	.003	21.164	82.612
		5	27.270	15.992	.116	-7.929	62.468
	3	1	-15.176	13.959	.300	-45.900	15.548
		2	-51.888(*)	13.959	.003	-82.612	-21.164
		5	-24.618	17.449	.186	-63.023	13.786
	5	1	9.443	15.992	.567	-25.756	44.641
		2	-27.270	15.992	.116	-62.468	7.929
		3	24.618	17.449	.186	-13.786	63.023
MFA(ave)	1	2	13.857(*)	2.971	.001	7.318	20.395
		3	9.742(*)	3.430	.016	2.192	17.292
		5	10.575(*)	3.930	.021	1.926	19.225
	2	1	-13.857(*)	2.971	.001	-20.395	-7.318
		3	-4.115	3.430	.255	-11.665	3.435
		5	-3.282	3.930	.421	-11.931	5.368
	3	1	-9.742(*)	3.430	.016	-17.292	-2.192
		2	4.115	3.430	.255	-3.435	11.665
		5	.833	4.288	.849	-8.604	10.271
	5	1	-10.575(*)	3.930	.021	-19.225	-1.926
		2	3.282	3.930	.421	-5.368	11.931
		3	-.833	4.288	.849	-10.271	8.604
mw mfa	1	2	8.906(*)	3.474	.026	1.259	16.553
		3	7.485	4.012	.089	-1.346	16.315
		5	8.295	4.596	.099	-1.821	18.411
	2	1	-8.906(*)	3.474	.026	-16.553	-1.259
		3	-1.421	4.012	.730	-10.252	7.409
		5	-.611	4.596	.897	-10.728	9.505
	3	1	-7.485	4.012	.089	-16.315	1.346
		2	1.421	4.012	.730	-7.409	10.252
		5	.810	5.015	.875	-10.228	11.848
	5	1	-8.295	4.596	.099	-18.411	1.821
		2	.611	4.596	.897	-9.505	10.728
		3	-.810	5.015	.875	-11.848	10.228

Based on estimated marginal means

* The mean difference is significant at the .05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Cluster Analysis:

Table 20 a: Final Cluster Centers for fibre traits

	Cluster		
	1	2	3
Crs(ave)	334.422	367.676	408.744
MFA(ave)	20.7126	21.7395	12.1331
mw mfa	16.1714	17.8418	11.8262

Table 20b: Distances between Final Cluster Centers

Cluster	1	2	3
1		33.312	74.941
2	33.312		42.603
3	74.941	42.603	

Table 20c: Number of Cases in each Cluster

Cluster	1	3.000
	2	9.000
	3	3.000
Valid		15.000
Missing		78.000

Further statistical data can be found in Appendix 3.

4.6 Discussion and Conclusions

Some wood and fibre properties that are characteristics of jack pine, lodgepole pine, and hybrids of these two species have been identified in Section 4.5. The objective of this chapter was to identify differences in wood and fibre traits among the species groups and provide for future prediction of fibre traits based on knowledge of species, or vise versa. Species groups were broken into 5 categories, as in Chapter 3, according to genetic results obtained in Chapter 2. Jack pine, lodgepole pine, and hybrids made up the first three groups; group 4 was composed of samples collected from the jack pine area that were genetically unique from other jack pine samples, and species group 5 was composed of samples collected from the hybrid area that showed up as genetic lodgepole pines. Species groups 4 and 5 were differentiated for the statistical purposes in Tables 12, 14, 16, 17, and 19, but left in regular sampling area groups for most of the comparison analysis; it was found that separation of these groups for visual comparison did not have a noticeable difference on conclusions.

4.6.1 Discussion of Solid Wood Properties

Average moisture contents, shown in Table 11, were variable depending on age of samples, site conditions, and specimen health. As a percent of oven-dried, and considering the whole core was used in the determination, the average moisture content (MC) of jack pine was the highest, while hybrids had the lowest moisture content. The pairwise comparison of moisture content revealed that significant differences exist among jack pine, lodgepole pine and hybrid groups. However, because MC is lowest in the hybrid groups, it is not a characteristic that displays the intermediacy of hybrids

between pure species. In order to verify this result, it is necessary to consider the variables that directly affect MC, to ensure that comparisons between samples are a direct result of species difference and not age, site conditions or tree health. Age differences that are expressed in Table 8 show that jack pine stands are much younger than lodgepole pine stands for this study. Hybrid stands are of intermediate age. A possible explanation for MC variation is as a tree ages, it gains a larger percentage of heartwood, which often has a lower moisture content.

Jack pine samples are the youngest, and possess the highest MC. However, lodgepole pine samples are the oldest and do not have the lowest MC, therefore factors other than age may be influencing MC. Since no obvious difference exists in the health of the trees sampled, it is logical that site conditions are the explanation for the variation in MC. Site indices are different for each site indicating small differences in nutrient and moisture regimes. The higher the site index value, the more suitable the site, i.e. the higher the nutrient regime and the more access to water sources the trees have. If this is the case, then it may be assumed that the jack pine sites were the wettest, because the jack pine samples had the highest MC's, while hybrid pine sites were the driest, because hybrid samples had the lowest MC values. Since jack pine sites were of the sandiest soil type, they were well drained and sites were probably drier than some with higher clay content; therefore site index may not have a direct relationship to wood MC, other site variables may provide more insight.

The time of sampling varied between sampling areas. Jack pine samples were not frozen when sampled in late September, lodgepole pine samples were partially frozen when sampled in November, and hybrid samples were completely frozen when sampled

in January. It is questionable whether or not this has an effect on the MC, but it is possible that frozen samples might be drier than samples that were not frozen when collected, due to the trees dormancy.

Average densities of the overall oven dried cores for each species are also listed in Table 11 and profiles of mature wood densities for each species are shown in Figures 27a, 27b, and 27c. Table 11 shows that lodgepole pine has the highest average density for the overall core, while hybrid pine has the lowest average density. The same results can be gathered from the density profiles of mature, earlywood in Figures 27a, b, and c. Although the average densities are different, only lodgepole pine is significantly different from the other species groups; hybrid pine and jack pine are not significantly different from each other with respect to overall average density according to the standard error comparisons in Table 11. The pairwise comparison in Table 12 indicates that no significant differences exist between jack pine, lodgepole pine or hybrid groups. Interestingly, only significant differences were found between species group 5 and the two pure species, which supports the conclusion in this study, that overall density is affected more by environmental influences than genetic influences, and cannot be a reliable characteristic for supporting genetic differences between species groups. Moreover, overall core density cannot be used to demonstrate intermediacy in hybrid samples.

A closer investigation of density with regard to mature wood exclusively, in Table 12, reveals that jack pine and lodgepole pine are significantly different and lodgepole pine and hybrids are significantly different. Jack pine and hybrids are not significantly different however, which suggests that hybrids are more like jack pine than lodgepole

pine when considering mature wood density. Like moisture content, mature wood density is a characteristic that is heavily dependent on outside factors. So, in order to determine if the variation in mature wood density is due to species type, and not variables such as site conditions, comparisons must be made between densities as a function of growth rate; a variable directly related to site conditions.

Individual core densities were investigated for their relationship to DBH growth rates in Figure 28a and b. Figure 28a shows the overall density of the whole core of all samples in relation to DBH growth rate. Figure 28b in contrast shows the densities of mature wood and earlywood only in select samples. It is expected that samples with a higher rate of growth would have a lower density, and samples with a lower rate of growth would have higher density. This is primarily due to a higher percentage of latewood in samples that grow slowly, and since latewood is denser than earlywood (Panshin and de Zeeuw, 1980), the sample is denser. Figure 28a and b show that jack pine samples and lodgepole pine samples were similar in their relationships between density and DBH growth; lodgepole pine had a lower growth rate and higher density, where as jack pine had a higher growth rate and lower density on average. Hybrid samples were slightly different in that they had a lower growth rate than jack pine samples, but a similar average density. Therefore even though hybrids were growing slower, perhaps due to site conditions, this does not increase their densities (or latewood percentage) as high as it would in lodgepole or jack pine samples. This suggests that latewood in hybrids may be less dense than in pure species, or that hybrids may form less latewood, in relation to earlywood, than pure species.

Table 13a, b, and c, show the results of a cluster analysis for all samples using the variables moisture content and density. These clusters were formed to maximize the variability between clusters. According to the majority of samples clustered, hybrid samples and jack pine samples are significantly different; half the lodgepole pine samples are similar to hybrids, while the other half of the lodgepole pine samples are more like jack pine samples. Very few of the samples were categorized into the third cluster, indicating that really only two groups can be identified as significantly different according to moisture content and density (see Appendix 3).

Earlywood : latewood ratios were calculated in this study in order to acknowledge the differences in the wood produced early in the growing season, with wood produced later in the growing season. The ratio itself provides distinction between different kinds of wood, and using it in a relationship to other variables such as density or cell wall thickness can help to explain variability in results. Based on the results, there is a large distinction between pure species and hybrids when observing average EW:LW ratios (Figure 29). The ratios for hybrid pine are much higher than both lodgepole pine and jack pine, suggesting that there is a higher percentage of earlywood present in the hybrids. Earlywood and latewood percentages are indicative of other characteristics because of their vast differences. Latewood contains smaller tracheids, with thicker cell walls and is higher in density than earlywood (Panshin and de Zeeuw, 1980). The relationship to cell wall thickness is demonstrated in Figure 31a, where an increase in the percentage of earlywood in a sample leads to a decrease in cell wall thickness, corresponding to evidence that earlywood cell walls are thinner than latewood cell walls.

A similar relationship may be assumed to exist between EW:LW ratios and density. Density increases with increases in cell wall thickness, therefore an increase in the percentage of earlywood in a sample would mean a decrease in the density of that sample. The percentage of EW to LW in a sample may be partially due to genetic inheritance within species. If factors such as age of samples and site conditions were controlled, it may be possible to support between species variation based on EW/LW %. In a study by Ivkovich et al. (2002), LW % in spruce was observed to have high heritability in both East Kootenay and Prince George study sites. This observation suggests that EW:LW ratios could be used as a species or population identifier. However, the pairwise comparison conducted in this study, shown in Table 14, revealed that no significant differences exist between the two pure species, or between jack pine, lodgepole pine, and the hybrids. This characteristic may be more valuable for distinguishing spruce species as indicated by Ivkovich's study (2002) due to the slower transition between earlywood and latewood in spruce as opposed to the abrupt transition in pines (Panshin and de Zeeuw, 1980). From the discussion it can be concluded that for this characteristic, hybrids did not demonstrate intermediacy between pure species.

Figures 30a and b are electron microscope pictures that display a) the ring profile of a hybrid sample, and b) the cross section of a jack pine sample. The ring profile gives an example of the transition from earlywood to latewood in pines, and also illustrates the percentage of earlywood to latewood that can be found in an average hybrid sample. The latewood reveals thicker cell walls than the earlywood; cell lumens are large and visible in the earlywood, but not in the latewood. The cross section of magnified cells in Figure 30b illustrates cell wall thickness, and cell dimensions. A wood ray is visible in the

center of the electron micrograph, and pits can be seen connecting tracheids, in the top, left corner of the picture.

Cell size is illustrated in Figure 31b. Lodgepole pine and jack pine cells have similar sizes with differences being that lodgepole pine cell diameter is larger in the tangential direction and jack pine cell diameter is larger in the radial direction. Hybrid samples seem to be of intermediate size in both directions; however differences in cell size are not significant enough to be considered a method of distinguishing between species groups.

Cell wall thickness was discussed previously in its relationship to EW: LW content. This can be further illustrated by Figure 32, which shows a profile of mature wood wall thickness moving towards the bark of the tree. Wall thickness values that are higher represent the latewood component of the growth ring, and lower wall thickness values represent the earlywood component. On average, lodgepole pine cell wall thickness is approximated at 3.02μm (Forintek, 1994), which is a representative value and because it is an average it does not incorporate the variation that is seen in Figure 32. The SilviScan values obtained from this study indicate that average cell wall thickness for jack pine is 2.43 μm, for lodgepole pine is 2.7 μm, and for hybrids is 2.4 μm. The lodgepole pine samples have higher cell wall thickness values than the others, and the hybrid samples have the lowest cell wall thickness value. The pairwise comparisons of each species group (Table 14) showed that the average cell wall thickness is significantly different between jack pine and lodgepole pine, and between lodgepole pine and hybrid pine, but not between jack pine and hybrids, suggesting that hybrid average cell wall thickness is more like that of jack pine rather than lodgepole pine. Cell wall thickness can therefore

be used to distinguish between some samples, but not others, and can not be used as an indicator of intermediacy in hybrids.

The relationship between cell wall thickness and density is expressed in Figure 33. As cell wall thickness increases, density increases. This is intuitive because the more cell wall material that is present in a measured area of wood, the denser that area would be, forming a direct linear relationship (Panshin and de Zeeuw, 1980). This relationship is evident in every one of the samples measured, and little difference exists between species groups, Figure 33.

Figure 34 shows the relationship between cell wall thickness and modulus of elasticity (MOE). No strong linear relationship is present between these two variables due to the large cell wall thickness variations from earlywood to latewood, but based on this figure and Figure 35, differences can be distinguished between species groups with regard to MOE. Lodgepole pine has the highest MOE, jack pine has the lowest, and hybrids show intermediate MOE. According to Table 14 differences in MOE are significant between all species groups except group 3 (hybrids) and species group 5 (samples from hybrid sampling area with lodgepole pine genetic output). Therefore, modulus of elasticity is a characteristic that can distinguish between species groups in this study, and can be used to indicate intermediacy of hybrids. In order to extend this into a general statement regarding distinction between lodgepole pine, jack pine, and hybrids outside of this study, factors including site conditions and sample age must be considered. The results of MOE also support the theory that species group 5 are in fact hybrids but may have lost jack pine genetic material over generations of back-crossing, these groups do not display significant variation in MOE.

Figure 37 shows the relationship between MOE for mature wood only, which addresses the issue of age difference, and growth rate according to DBH, which is a direct reflection of site conditions. Figure 37 shows that there is a distinction between species groups. Jack pine has a lower MOE on average, which is associated with a higher growth rate. This is an expected trend, since as trees grow faster, they produce wider rings that are less dense and the stems contain more juvenile wood (Panshin and de Zeeuw, 1980). Therefore, wood has lower MOE. Lodgepole pine has a lower growth rate and higher MOE. Hybrids are intermediate with similar growth rates to lodgepole pine but lower MOE. Since this figure reflects the same results as those found in Figure 35, a difference in MOE between species groups is due to genetic variation and therefore can be extended to the pine populations in these areas.

Tables 15a, b, and c reflect the cluster analysis preformed in order to group samples together based on mw density, EW:LW ratio, MOE, and cell wall thickness. Since only five samples from each of the pure species groups, 3 samples from the hybrid group, and 2 samples from species group 5 were analyzed for these characteristics, the cluster analysis is limited in its statistic representation. However, cluster formations do indicate that some samples are more alike than others, with respect to these characteristics. The samples forming cluster 1 consist of 50% jack pine samples. Cluster 2 is made up of 100% lodgepole pine samples, and cluster 3 contains 50% hybrid samples. Even though there is not an overwhelming trend separating the three main species groups from one another, only one species groups occupies the majority of each cluster, indicating that there is a possibility of species distinction based on these characteristics if more samples were tested.

In order to better support the conclusions drawn from the wood analysis portion of this study, alternate characteristics could be looked at to achieve greater confidence in the variability between species. It was suggested in a study by Christensen and Dar (2003) that number of resin ducts can be distinctive of species. This could be further investigated and related to percentage of EW and LW in this study, in order to draw more conclusive evidence of differentiation between species groups and intermediacy of hybrids.

4.6.2 Discussion of Fibre Properties

Fibre properties are directly correlated to several wood characteristics that have already been identified. Fibre length and microfibril angle are both a reflection wood maturity (Barbour, 2004), and fibre coarseness is directly correlated to cell wall thickness, and therefore EW:LW ratio (Potter et al., 2001). Although relationships exist between these characteristics, differences can be noted in their significance for differentiation between species groups investigated in this study.

Figure 38 shows the length weighted fibre length from pith to bark in all samples. The relationship between fibre length and wood as it matures is represented well. It shows that as wood matures from pith to bark, fibre length increases until a pure mature wood stage is reached at which point the increase levels off and fibre length becomes relatively constant. Lodgepole pine has the greatest fibre length at all stages of wood development (Figure 38). Jack pine fibre length is shown to be intermediate, and hybrid fibres are shown to be the shortest of the three species groups. Species groups 4 and 5 were not separated out for this analysis. As explained earlier, little variation occurred in

conclusions drawn from sample separation. According to Figure 38, it can be concluded that there is a difference in fibre length among species groups, but hybrids do not display intermediacy between the two pure species. Even though Figure 38 presents evidence of species differentiation, it is important to consider influence of site conditions and statistical reliability.

The number of fibres sampled per fibre size interval is reflected in Figure 39. There is a large sample size represented here, which enhances the reliability of the data, based on which a pattern of fibre lengths can be differentiated. There is a larger amount of short fibres in jack pine, and a larger number of longer fibres in lodgepole pine. The largest number of fibres in hybrids is intermediate of the two pure species. Even though the sample size considered here is large enough for statistical reliability, the pairwise comparisons conducted in Table 16 and 17 show that there are no significant differences between any species group with respect to fibre length at any stage of wood maturity. Therefore fibre length can not be used as a discriminating characteristic between species groups or support intermediacy in hybrids; however, the fibre length distribution curve (Figure 35) is useful for displaying intermediacy of hybrid fibre lengths.

Another characteristic investigated in this study is fibre coarseness, and is largely dependent on fibre length (Figure 40). Lodgepole pine samples have the largest fibres, both in length and coarseness. Jack pine and hybrid samples have shorter fibres compared to lodgepole pine, and are made distinctive of each other based on coarseness. Hybrid samples are slightly coarser than jack pine samples. Lodgepole pine samples are coarser than hybrid and jack pine samples from 40-80 years from the pith (Figure 41). Table 16 shows that the difference in coarseness between jack pine and lodgepole pine is

significant, as well as the difference between lodgepole pine and hybrids at 40-60 years from the pith. At 60-80 years from the pith there is a significant difference between lodgepole pine and hybrids, but no difference between jack pine and the hybrids or lodgepole pine samples. There is no significant difference between the fibre coarseness of samples at 20-40 years from the pith (Table 17). With that, it is possible to use coarseness to distinguish between lodgepole pine and jack pine, and lodgepole and hybrids, and therefore hybrid coarseness values may be more closely related to jack pine samples than lodgepole pine samples. On the other hand, average overall coarseness in Table 19 displays that there are significant differences between jack pine and lodgepole pine, and jack pine and hybrids, indicating that hybrids are more closely related to lodgepole pine. Due to this discrepancy, a greater sample size should be used in the overall coarseness analysis (Table 19), because only 15 separate samples were measured at multiple positions for the overall measurement by SilviScan. Coarseness profiles in Table 16 are from FQA analysis of over 80 samples and therefore reflect a more accurate representation. Coarseness does not indicate intermediacy in hybrids by either calculation. Figure 42 investigates the relationship between coarseness and density. This figure shows a direct correlation, where an increase in coarseness leads to an increase in density. Lodgepole pine samples are have coarser fibres, and are denser; hybrid fibres are the finest and samples are not as dense. Coarser fibres therefore lead to denser wood, which is usually wood that has grown more slowly, and developed a higher percentage of latewood (Panshin and de Zeeuw, 1980).

Table 18a, b, and c show the cluster analysis results for fibre length and coarseness. No conclusive results are reflected from this analysis with respect to species groupings.

Clusters 1, 2, and 3 all contain samples from all three species groups. Based on these results, some samples can be seen as more related to hybrids than others, but it is not dependent on species group.

Microfibril angle was investigated in order to determine its ability to distinguish between species groups. Figure 43 shows the profiles of each sample from pith to bark with respect to MFA, and average MFAs over the whole core is displayed in Figure 44. Jack pine samples show a profile with greater MFAs and lodgepole pine samples show profiles with lower MFAs. Hybrid samples show MFA profiles with intermediate values. These relationships are reflected almost continuously over the whole profile, indicating that even mature wood displays differences in MFA between species groups; differences are not only due to juvenile wood percentage or the variation in age of sample. The same trend is observed in both Figure 43 and 44. Therefore, it can be assumed that jack pine has higher MFAs in general than lodgepole pine, and hybrids display intermediacy for this trait. In order to verify this trend, and ensure that variation in MFA is not due to site conditions, MFA versus growth rate is shown in Figure 45. This relationship reveals that some jack pine samples have faster growth rates corresponding to higher microfibril angles. However, other jack pine samples reveal the same MFAs as lodgepole pine or hybrid samples for varying growth rates, indicating that growth rate does not have an affect on the MFA of every sample in this study. Table 19 displays statistical significance in a pairwise comparison for both mature wood MFA and overall MFA. For overall MFA, jack pine is significantly different from all samples, all other samples are not significantly different from each other. Mature wood MFA, which is a more accurate way to compare species groups as it is not dependent on age, reveals that jack pine and

lodgepole pine are significantly different from each other. However, hybrids are not significantly different from either species group.

Considering all relationships, MFA can be used as a characteristic to distinguish between jack pine and lodgepole pine pure species, but not hybrids. However, MFA can be used to support the theory of intermediacy in hybrids. To add to this, the relationship between MFA and MOE is shown in Figure 46, which can give insight into using MOE to predict MFA, or vise versa.

Table 20a, b, c show the results of a cluster analysis preformed to determine how samples can be grouped when considering influences of overall coarseness, overall MFA, and mature wood MFA. Four out of five jack pine samples fell into cluster 2, three out of five lodgepole pine samples fell into cluster 3, and three out of five hybrid samples fell into cluster 2. This indicates only that hybrid samples may be more related to jack pine samples than lodgepole pine samples. However, considering that only five samples were available for clustering per species group, it would be more statistically reliable to study a larger sample size in the future.

4.6.3 Conclusions

In summary, several wood and fibre quality traits are useful in combination to help identify species groups and support hybridization. Moisture content and MOE were the most valuable characteristics for differentiating between pure jack pine, lodgepole pine, and hybrids of the two species in this study, as well as acting as traits to support the theory of intermediacy in hybrids; hybrids show intermediate traits of the pure species they are derived from. In addition to these characteristics, cell wall thickness and fibre coarseness can be used to differentiate between lodgepole pine and jack pine, and

lodgepole pine and hybrids. Because these traits indicate no significant difference between jack pine and hybrids, the hybrids have characteristics like jack pines. Finally, fibre length distribution and MFA, although not statistically different for hybrid differentiation from pure species, do show intermediacy of hybrid characteristics, further supporting this theory.

Possible sources of error that may have skewed results include site conditions unaccounted for such as stand density and position, wood types such as reaction wood that may have be present in the stems, and any undiagnosed disease or pest attack. These variables were controlled as well as possible with factors such as growth rate and site index taken into consideration. The objective of this Chapter, to compare species based on wood and fibre traits in order to identify if any significant differences, was met; results indicate that some traits are more distinctive than others for differentiation of hybrids in the Fort Nelson region of British Columbia.

4.7 References

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Chapter 5: Chemical Analysis of Jack Pine, Lodgepole Pine, and their Hybrids

5.1 Chapter Objectives

The objective of this chapter is to determine if a "chemical extractive footprint" can be identified for each of the three species groups: jack pine, lodgepole pine, and hybrids, and to determine if this footprint can be used to differentiate between species. The creation of an extractive footprint by gas chromatography – mass spectrometry enables the identification of some of the extractives within wood that are characteristic to one or more of the species groups in question, or allow for characteristic quantities of the same extractives to be identified within a species group. Characterizing the species in this way allows for further support of hybrid existence, and support the morphological evidence of hybridization that is prominent in scientific literature and found in Chapter 3.

5.2 Wood Chemical Extractives from Literature

Wood extractives usually account for less than 5% of wood volume and are not considered a structural wood component. Wood extractives can be categorized as triglycerides, resin acids, fatty acids, terpenoids, waxes, phenolic acids, or others, depending on the literature. These compounds are found throughout the wood at different locations depending upon the chemical (Sjöström, 1993). Large amounts of chemical extractives are found in the cell lumens, but extractives can also be found in the cell walls (Higuchi, 1985). Resin acids are found within the resin ducts and fatty acids are found in the ray parenchyma. Most species have a distinct pattern of chemical extractive types, amounts, and locations, although similar within families (Sjöström, 1993).

Functions of a cell within a specific species are detectable based on the chemical extractive makeup found within the wood. Fats provide energy for the cells, and terpenoids, resin acids, and phenolic acids aid in defense mechanisms to protect the tree from pests and pathogens (Sjöström, 1993). Therefore, if a tree has particularly high levels of resin acids, we may be able to assume that the species has a need to defend itself, and therefore has a larger percentage of pests and pathogens that can harm it compared to another species with lower resin acid levels. Links can then be made from this idea to environmental and biological reasons that the tree is more susceptible to attack, such as thinner bark, or favored climate or elevation range.

Chemical extractive footprint can be used to identify similarity between species. In this case, determining the differences, if any, between lodgepole pine and jack pine will aid in the identification of a possible hybrid between these two species.

The ratio of extractive and tissue amounts varies between species and families and is therefore the basis of chemotaxonomy that is, to identify plant material based on chromatographic output. Polyphenol compounds are most commonly used for chemotaxonomy due to their distinctive nature, and have been used to group species in *Pinus*, *Prunus*, *Acacia*, and *Eucalyptus* genera (Higuchi, 1985).

The amount of extractive varies widely between species, and have extensive effects on wood quality. For example, extractives can be a hindrance in pulping processes, and can affect wood color in furniture or instrument manufacturing (Higuchi, 1985).

Terpenoids are composed of hydrocarbons and their derivatives with over 7500 different structures divided into subgroups according to isoprene unit; hemi, mono,

sesqui, di, sester, tri, tetra, and poly prefixes indicate the number of carbon and isoprene units found in the terpene compound (Sjöström, 1993).

Softwoods house fats and waxes in ray parenchyma while hardwoods contain fat and wax abundantly throughout all parenchyma. More than 30 fatty acids have been identified in softwoods and hardwoods; the most common unsaturated fatty acids are oleic and linolenic acid and pinolenic acid is a major fat in pines and spruces. Waxes can be categorized as free fatty alcohols, esters of higher fatty acids, or terpene alcohols (Sjöström, 1993).

Phenolic compounds are a large category of extractive chemicals also known as complex aromatic extractives. Compounds in the phenol family can be further broken down into subgroups; stilbenes, lignans, hyrolyzable tannins, flavanoids, and condensed tannins. Typical phenolic compounds found in pines are the flavanoid known as chrysin, the stilbene known as pinosylvin, and the lignan, pinoresinol. Hyrdolyzable tannins are uncommon in wood and condensed tannins occur mostly in hardwood barks (Sjöström, 1993).

A small percentage of the extractive component of wood is composed of inorganic compounds (<1%). Such inorganics include: silicates, oxalates, phosphates, calcium, potassium, magnesium, iron, cobalt, and manganese (Sjöström, 1993).

5.3 Experimental Design and Hypotheses

A core sample (pith to bark) from each tree was used for the acetone extraction, and chemical extractive makeup of each sample was determined using gas chromatography- mass spectroscopy (GC-MS).

Thirty samples of pure lodgepole pine, 30 of pure jack pine, and 30 potential hybrid samples were used for this analysis; from the Prince George area of BC, the Smoky Lake area of Alberta, and the Fort Nelson region of BC, respectively. Each site was chosen for its similarity in vegetation and moisture/nutrient regimes. Sites for lodgepole pine and jack pine were chosen as to be similar distances from the potential hybrid zone.

5.4 Methodology

5.4.1 Chemical Analysis Using GC-MS

Gas chromatography-mass spectroscopy (GC-MS) was used to analyze the chemical extractive components of the wood cores. The GC uses helium gas moving through a capillary column 10 meters long and only 0.25 mm in diameter to separate each chemical component individually for analysis. As each chemical moves though the column, it either has an affinity for the column or an affinity for the helium. Depending on its level of affinity for the stationary phase (the column) or the mobile phase (the helium), which is generally individually distinctive, the chemicals gradually separate. This separation means the chemicals move through the column at different speeds and therefore a retention time, how long the chemical spent in the column, can be measured. Chromatograms are created based on these retention times, each chemical substance or component represented by a peak at the specific retention time measured. The peak size or area represents the amount of chemical present, or the strength of the measurement.

Once the substances are separated through the column, and retention times are recorded, the substances move into the mass spectrometer (MS), where they are analyzed. The MS consists of an electron beam which comes in contact with each

chemical and forces ions to break off, which are counted in an ion trap. This allows the chemical mass to be identified based on how many ions of the substance are present in relation to each other, and gives a spectral analysis output which contains the chemical mass, the "parent ion" which is the chemical or chemical component most represented in the mass spectra, and other ions, that may be other chemicals present in the substance or degraded chemical constituents.

Chemical Extraction Protocol

Cores were oven-dried and weighed. Two-hundred milliliters of acetone was measured into round bottom flasks. Each sample was individually extracted with the acetone using a heating and cooling apparatus to cycle the acetone and draw chemicals from the wood over a five hour period.

The 200 ml of acetone plus extractives was then condensed down to 5 ml of liquid using a Rotavapor® heating, cooling, and vacuum system, or condensed down to 5 ml of liquid using a TurboVap® with heat and nitrogen gas.

After the acetone solution was brought to a volume of ~5 ml, the liquid was poured into small pre-weighed vials. The vials were then placed under a low heat and nitrogen gas to blow down the remaining liquid to approximately 1ml, composed of chemical extractives from the wood samples while minimizing loss of volatile extractives. Vials were then sealed with Parafilm®, and placed in a -86^{0}C freezer. Once samples were frozen they were freeze dried.

Dried samples were weighed to determine the percentage of the original core that was extracted. The dried samples were then re-suspended at 5.000 mg/ml, in an internal standard solution of Ethyl acetate containing 0.250 mg/ml heptadecanoic acid.

One microliter of each sample was then programmed into the GC using a run-time of 42 minutes.

Chromatography- Identification and Quantification of Compounds

Extractive profiles (mass spectral output) were established after MS was completed. Each chemical profile was composed of a series of peaks, representing how the ions were broken during the MS.

Chromatographs were aligned subsequent to initial output, based on the internal standard used, in order to properly compare the peaks between samples. After alignment the chromatographs were still subject to slight differences due to stretch. This variability is due to the GC analysis; it is very difficult to maintain exactly the same conditions within the column being utilized. Because of this "stretch" variability, the chromatographs were used to form 20 peak regions for analysis, instead of identifying each individual peak. Therefore it was possible to compare regions between samples, without having to be concerned that any between sample variability was caused by stretch in the chromatogram. These peak regions were analyzed using partially least squares discriminant analysis (PLS-DA) in order to identify regions that were the most variable between species. The peak regions that caused the most variability between species groups were then identified by mass spectra and compared to a mass spectral library where extractive profiles are matched to the closest possible found in the library or authentic known standards. If compounds did not return an appropriate match, the

chemical substance was classified based on broad spectral features. The library returns chemical matches based on the purity of the samples, the fit, and the retro-fit. These identifiers have a maximum value of 1000, which would indicate a perfect match. Purity indicates the number of extra peaks that occur in the sample as compared to the candidate substance from the library. The fit indicates how closely the sample mass spectra fits with the mass spectra in the library, and the retro-fit indicates how closely the library mass spectra for the candidate substance fits with the sample mass spectra.

The 20 peak regions analyzed by PLS-DA were also used to create a discriminant variable with which to compare the samples. The discriminant variable was used to identify how alike the samples were to lodgepole pine.

5.5 Results

Table 21: Average % extractives per sample for each sample site.

	% Extractives
Jack pine site 1	4.466
Jack pine site 2	4.826
Jack pine site 3	2.456
Lodgepole pine site 4	1.691
Lodgepole pine site 5	2.580
Lodgepole pine site 6	4.125
Hybrid pine site 7	4.152
Hybrid pine site 8	4.815
Hybrid pine site 9	1.884

Table 21 show the average amount of extractives that were found in each sample according to collection site (%).

5.5.1 PLS-DA Output

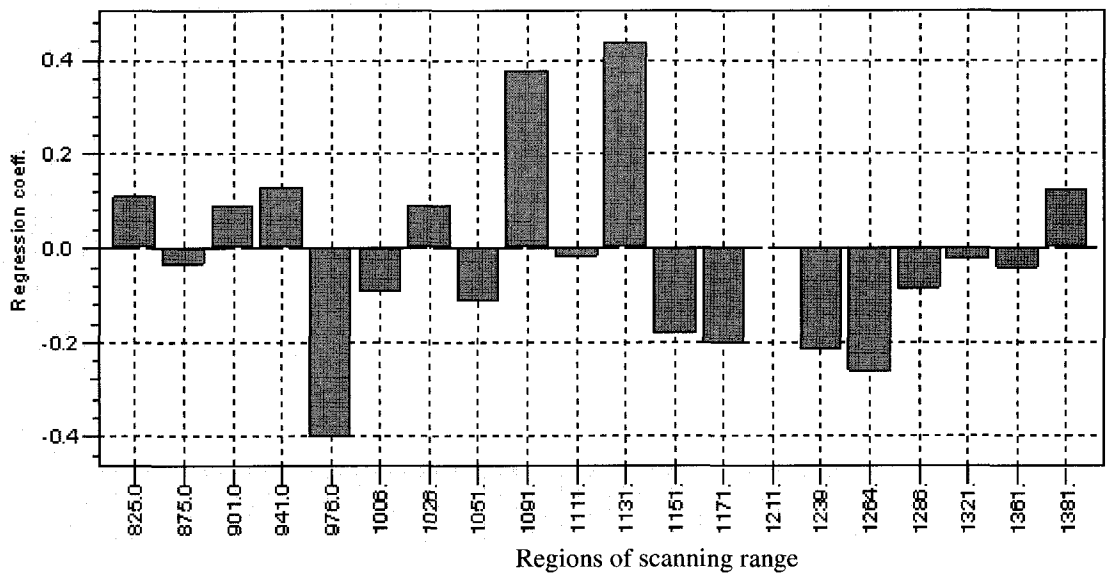


Figure 47: PLS-DA model output of the regression coefficient for lodgepole pine. The greater the coefficient value, the greater the contribution of that peak region to the variability between the sample groups. A negative coefficient value indicates a negative correlation to lodgepole pine.

Chromatogram regions are indicated on the x-axis of Figure 47. These regions were used in order to eliminate variability due to stretching in the chromatographs. There are several peak regions on the chromatograms that contribute to the variability between samples based on chemical analysis (Figure 47). Out of the 20 regions indicated, 3 are responsible for a large amount of variability, represented by regression coefficients greater than +/- 0.28. These 3 regions showing the most variability are region 5 (representing scan range from 975-1005), region 9 (representing scan range from 1090- 1110), and region 11 (representing scan range from 1130-1150) and are shown on the chromatogram shown in Figure 48. Region 9, at 1131, indicates the most variability between sample groups. Region 5, at 976, is responsible for the second highest amount of variability between groups, and region 11 is the coefficient showing the third highest variability.

5.5.2 Chromatography

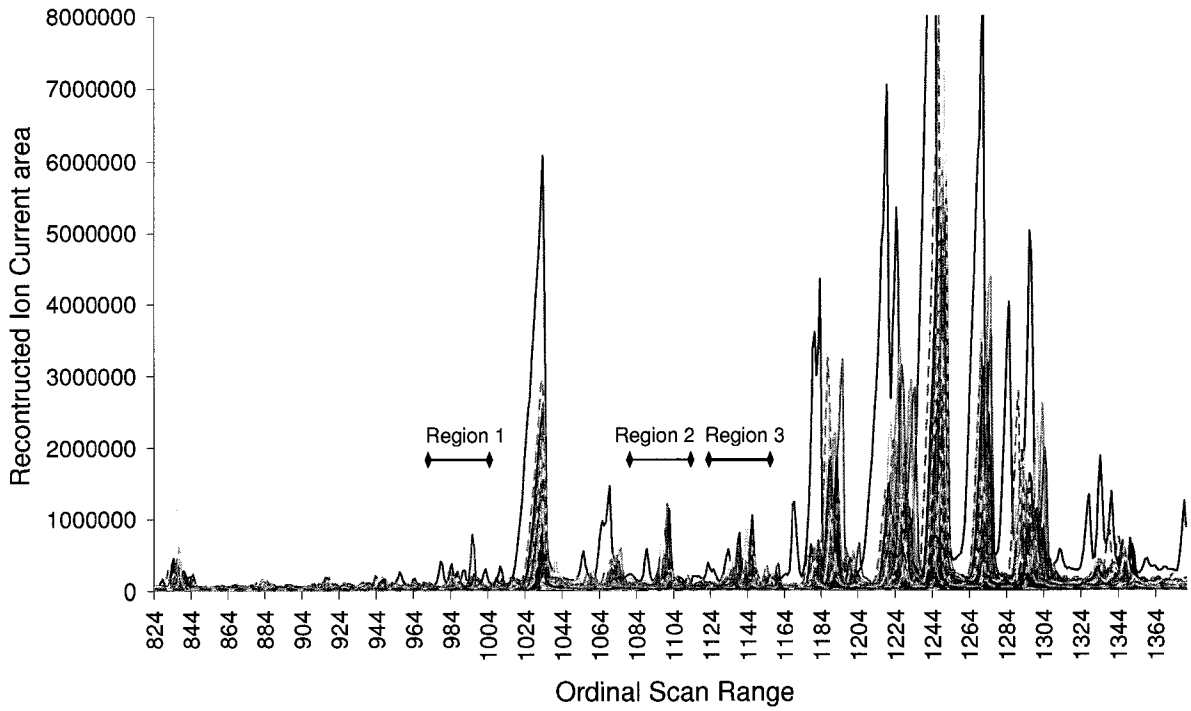


Figure 48: Aligned Chromatogram for range 825-1380, where most variability in peaks was determined to be. Regions of variability according to the PLS-DA output are indicated.

The chromatogram shown in Figure 48 shows the regions that are responsible for the most variability between species groups according to the PLS-DA conducted and displayed in Figure 47. Within these regions, main peaks were identified in order to analyze the potential extractive chemicals by mass spectrometry. More than one main peak is identifiable in regions 5 and 11, and region 9 has one main peak for analysis. These peaks were all analyzed by mass spectra in Figures 50a-f.

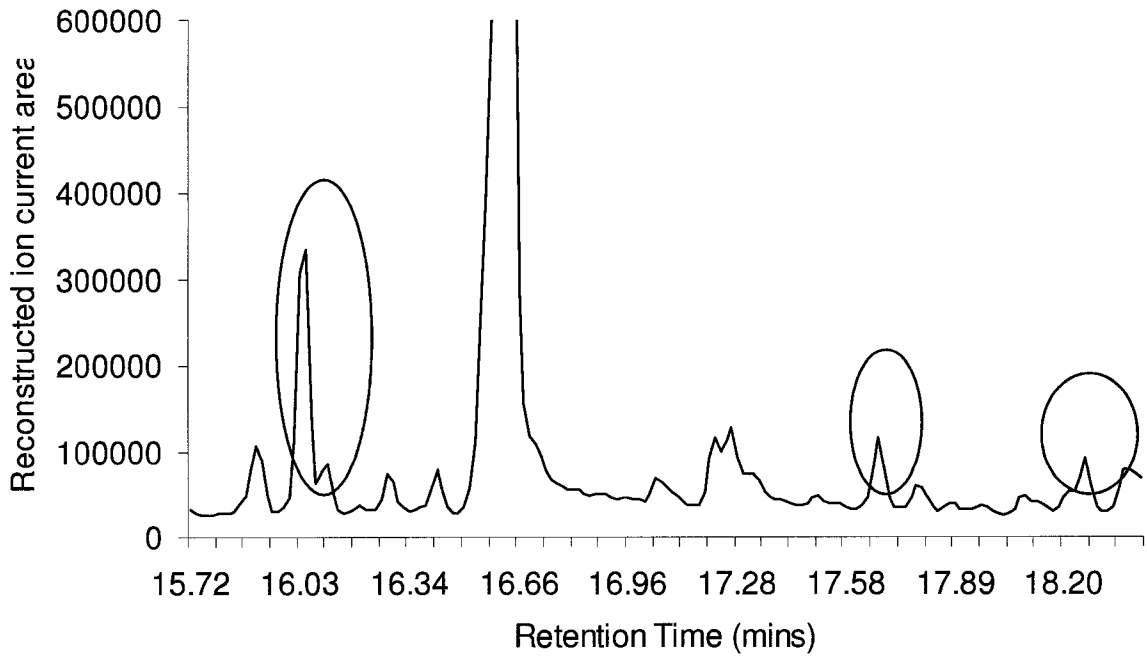


Figure 49a: Chromatogram of jack pine sample 2. Peak regions used for PLS-DA are highlighted by circles.

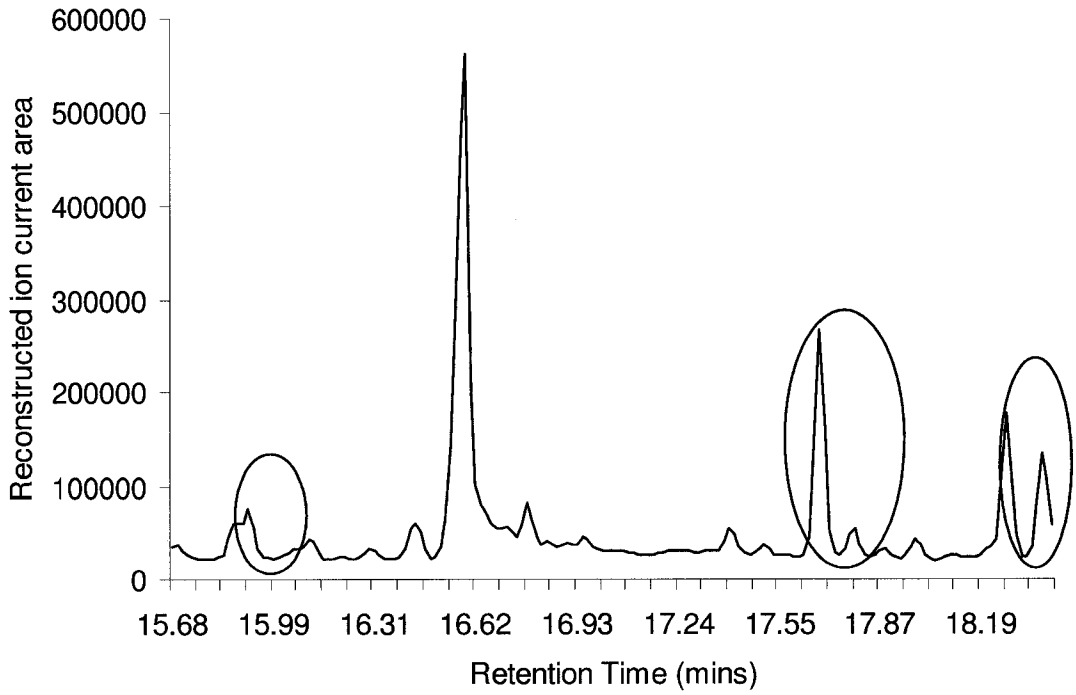


Figure 49b: Chromatogram of lodgepole pine sample 43. Peak regions used for PLS-DA are highlighted by circles.

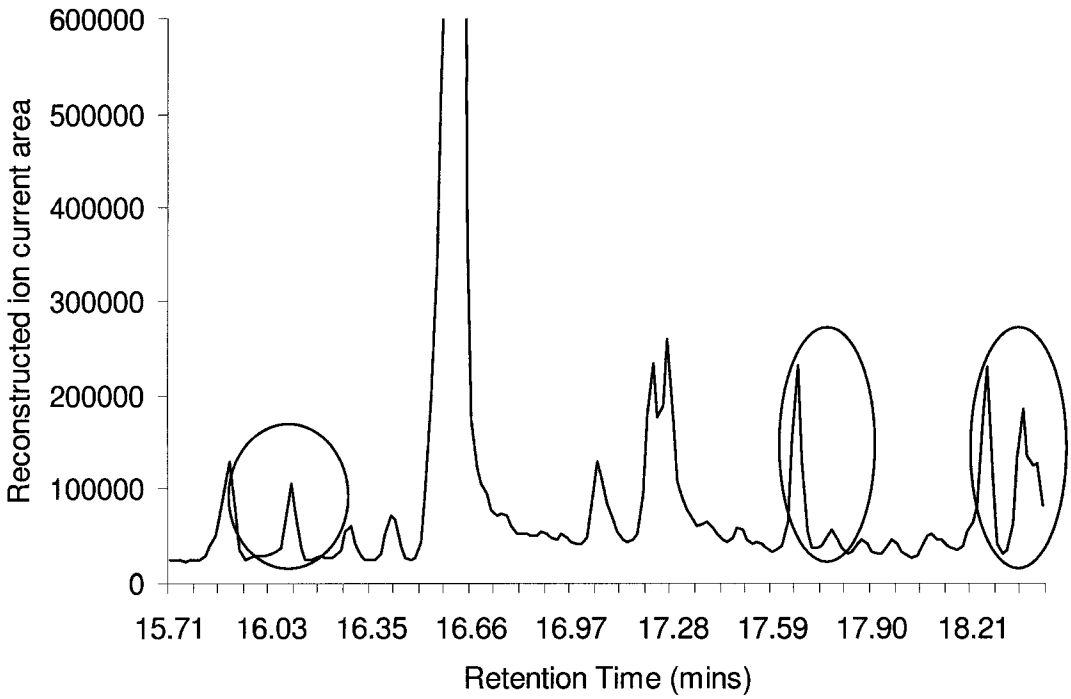


Figure 49c: Chromatogram of hybrid pine sample 68. Peak regions used for PLS-DA are highlighted by circles.

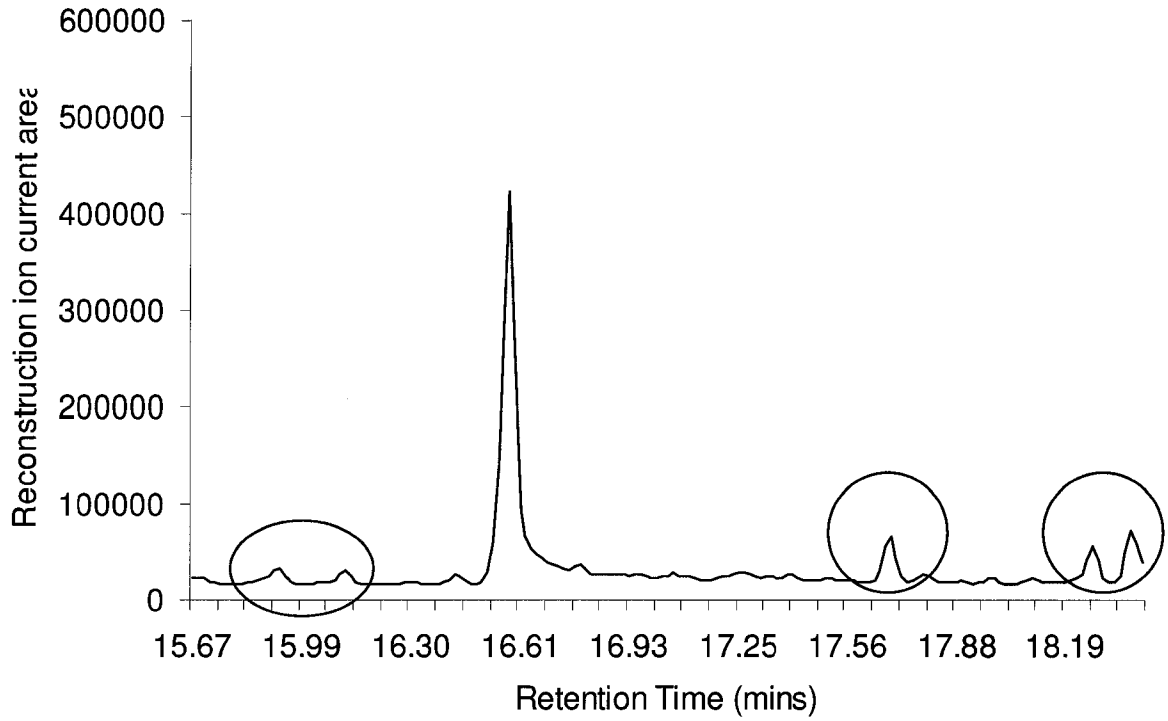


Figure 49d: Chromatogram of hybrid pine sample 84. Peak regions used for PLS-DA are highlighted by circles.

Figures 49a-d show the chromatograms for single samples from each species group according to retention time. Figure 49a represents the typical output for jack pine samples (samples 1-30), Figure 49b represents the output for lodgepole pine samples (31-60), and Figure 49c is representative of hybrid samples (samples 61-80). Figure 86d is included also because it represents the samples that were collected from the hybrid sampling area but were unique possible Fn generation hybrids back-crossed with lodgepole pine, according to the genetic RFLP output (samples 81-90). The same comparisons can be made between Figures 49a and b as can be seen in the regression coefficient output in Figure 47. It is noticeable that peak region 5 (circled on the far left of the chromatograms) is much larger in Figure 49a (jack pine) than the others; region 9 (circled in the centre of the chromatograms) is larger in the lodgepole pine profile (Figure

86b); and region 11 is the largest in Figure 49c (hybrid samples), but larger in lodgepole pine than jack pine. The size of the peak indicates quantity of chemical present.

5.5.3 Mass Spectral Analysis

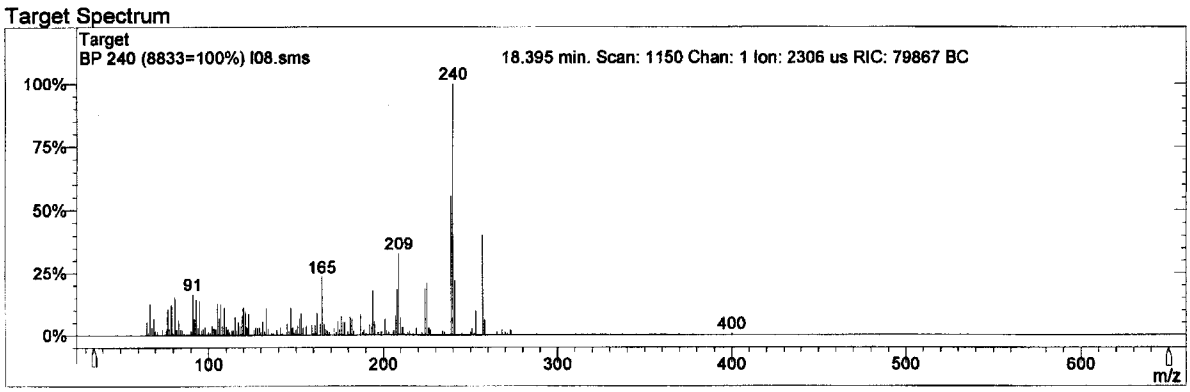


Figure 50a: Sample 08, peak region 11 at a retention time of 18.395 minutes. The parent ion is shown at a mass of 240 m/z.

Table 22a: Library search results of potential chemical compound matches for the mass spectra in Figure 50a.

	Purity	Fit	RFit	Entry #		MW.	Formula	Name
1.	470	729	528	91361 A	240		C16H20N2	2,6,2',6'—Tetramethyl
2.	450	715	504	91374 A	240		C16H20N2	4,6,2',6'—Tetramethyl
3.	444	692	476	19505 B	240		C16H20N2	[1,1'—Biphenyl]—4,4
4.	439	700	501	91313 A	240		C16H20N2	[1,1'—Biphenyl]—4,4'
5.	430	693	514	91368 A	240		016H20N2	[1,1'—Biphenyl]—4,4'—d
6.	419	665	545	91278 A		355	C16H29NO4Si2	Dimethoxymandelic
7.	409	721	443	91289 A	240		C16H1602, None	Dimethoxy—stilbene

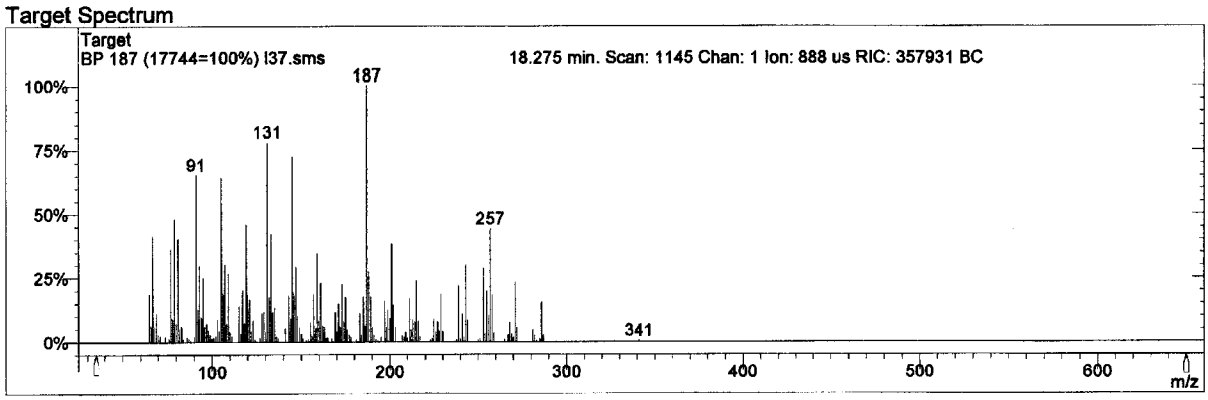


Figure 50b: Sample 37, peak region 11 at a retention time of 18.275 minutes. The parent ion is shown at a mass of 187m/z.

Table 22b: Library search results of potential chemical compound matches for the mass spectra in Figure 50b.

	Purity		Fit	RFit	Entry #		MW.	Formula	Name
3.	486	805	527	65379	A	376	C28H40		Cyclopentane-3'—spiropentacy
4.	474	755	539	19779	B	304	C20H3202		Methandriol
5.	464	841	501	12825	B	286	C20H300		Retinol
6.	440	773	501	37145	A	296	C22H32		Cyclodecacyclotetradec
7.	428	726	501	94121	A		272	C20H32	Phenanthrene, 7—etheny
11.	420	667	485	94122	A		272	C20H32	Naphthalene, decahydro—1
12.	419	730	523	30793	A	404	C30H44		Dibenzo[a,H]cyclotetra

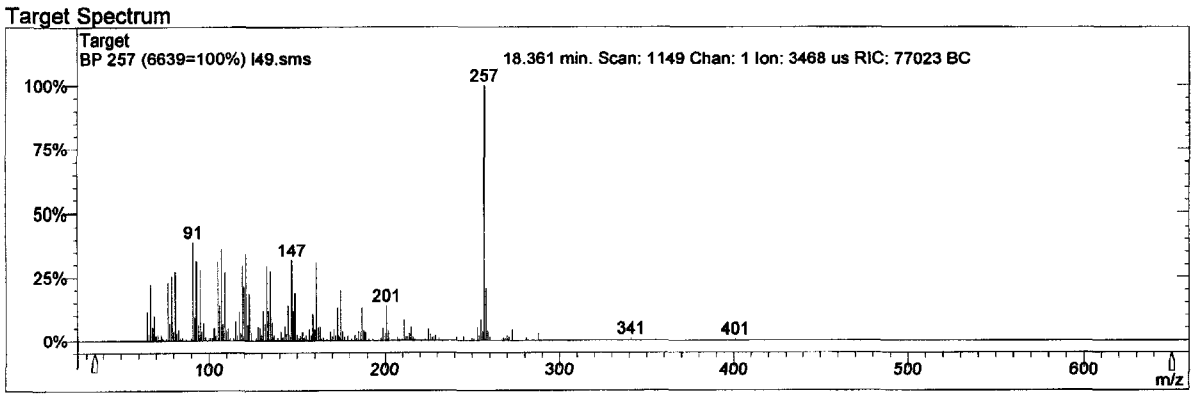


Figure 850c: Sample 49, peak region 11 at a retention time of 18.361 minutes. The parent ion is shown at a mass of 257 m/z.

Table 22c: Library search results of potential chemical compound matches for the mass spectra in Figure 50c.

	Purity		Fit	RFit	Entry #		MW.	Formula	Name
1.	661	821	749	4152	A		290	C20H340	Verticiol
2.	645	813	757	94122	A	272		C20H32	Naphthalene, decahydro—l
4.	638	827	729	94148	A	384		C28H48	Ergost—14—ene, (5.alph
5.	625	795	703	37678	A	406		C27H3403	19-Nor-4—androsten—3—one
6.	615	817	690	94144	A	370		C27H46	Cholest—14—ene, (5.alp
7.	577	762	664	39285	A	406		C27H3403	Nandrolone Phenpropiona

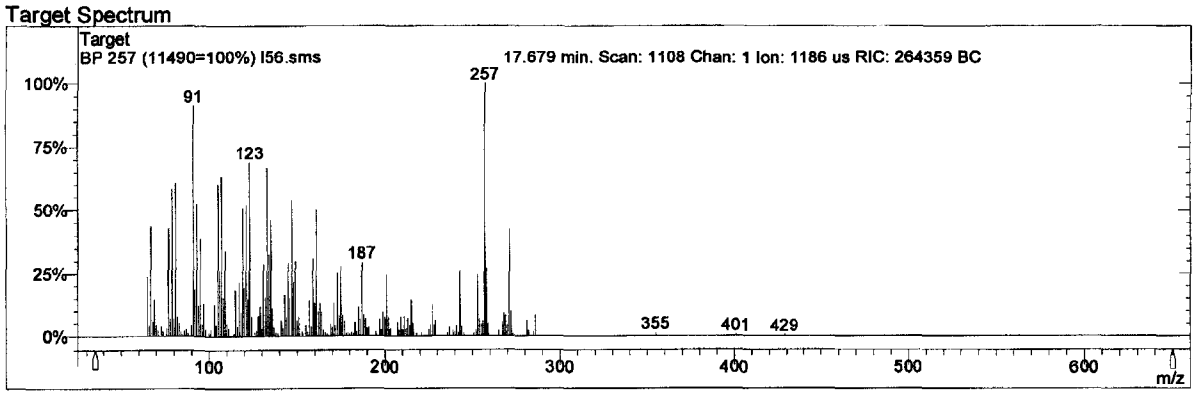


Figure 50d: Sample 56, peak region 9 at a retention time of 17.679 minutes. The parent ion is shown at a mass of 257 m/z.

Table 22d: Library search results of potential chemical compound matches for the mass spectra in Figure 50d.

	Purity	Fit	RFit	Entry #	MW.	Formula	Name
1.	653	847	695	94154 A	286	C20H300	1—Phenanthrenecarboxald"
2.	598	867	653	94152 A	290	C20H340	Verticiol
3.	574	817	623	94122 A	272	C20H32	Naphthalene, decahydro—1
4.	560	827	631	94121 A	272	C20H32	Phenanthrene, 7—etheny
5.	537	785	598	37678 A	406	C27H3403	19—Nor—4—androsten-3-one

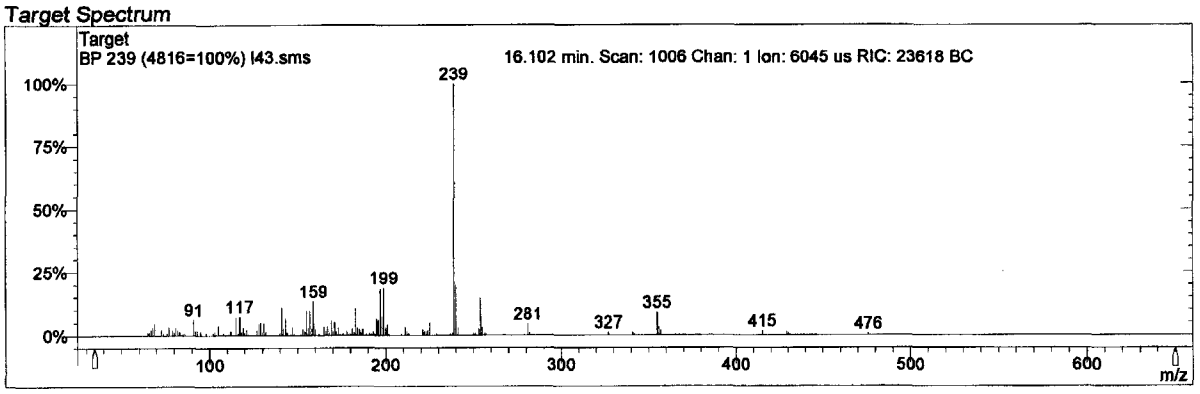


Figure 50e: Sample 43, peak region 5 at a retention time of 16.102 minutes. The parent ion is shown at a mass of 239 m/z.

Table 22e: Library search results of potential chemical compound matches for the mass spectra in Figure 50e.

	Purity		Fit	RFit	Entry #		MW.	Formula	Name
1.	523	718	685	91185 A		314	C21H3002		1—Phenanthrenecarboxy
2.	519	732	650	19478 B		314	C21H3002		1—Phenanthrenecarboxy
3.	515	743	651	91068 A		314	C21H3002		Phenanthrene—1—carboxylic e
5.	412	630	488	82546 A		240	C16H20N2		9—Methylene-l-phenyl—3,6—d
6.	409	737	493	91200 A		254	C19H26		Naphthalene, tris(1—me'
16.	374	832	379	91204 A		254	C16H1403		5—Hydroxy—6—methoxy—
23.	343	639	376	82013 A		254	C12H18O4Si		Benzoic acid, 3,4—d

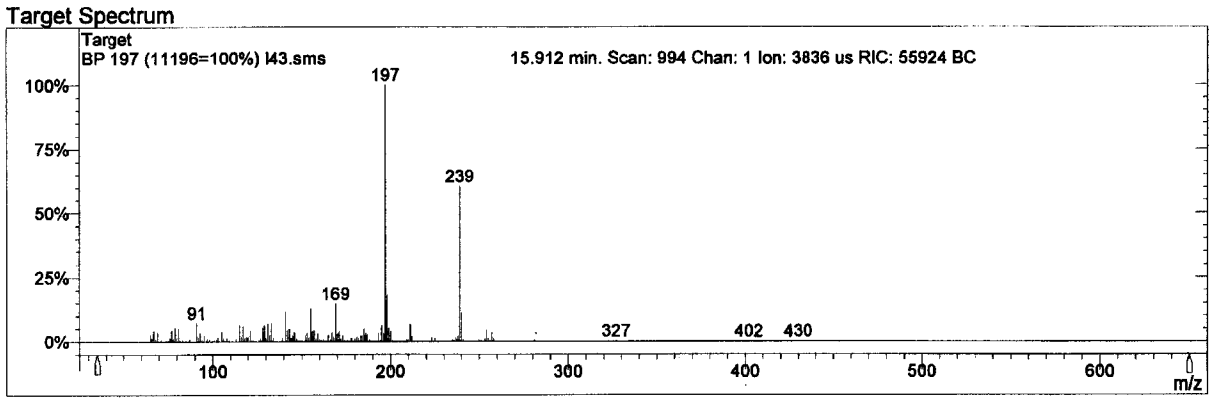


Figure 50f: Sample 43, peak region 5 at a retention time of 15.912 minutes. The parent ion is shown at a mass of 197 m/z.

Table 22f: Library search results of potential chemical compound matches for the mass spectra in Figure 50f.

	Purity		Fit	RFit	Entry #		MW.	Formula	Name
1.	559	662	677	19478	B	314	C21H3002		1—Phenanthrenecarboxy
2.	532	700	652	82546	A		240	C16H20N2	9—Methylene-l—phenyl--3,6—d
3.	499	594	641	91185	A	314	C21H3002		1—Phenanthrenecarboxy
4.	494	630	614	91068	A	314	C21H3002		Phenanthrene—1-carboxylic ***
5.	488	807	532	82415	A	254	C17H22Si		1—Tert—butyl—1—(naph——
6.	472	627	565	82514	A	254	C18H220		Spiro[cyclobutane—1,1
7.	467	580	527	91350	A	240	C18H24		Chrysene, 1,2,3,4,4a,4

Figures 50a-f show the mass spectra output for the peaks identified within the regions showing the highest variability between species groups. The largest ion percent in the mass spectral output is referred to as the parent ion. This varies between spectra, indicating differences between peak chemical compositions. The ions found with the higher molecular weights can provide indications of the molecular weight of the compound, as long as the peak is consistent throughout the samples.

The library output for each spectra indicates the name of the chemical that is a potential match, the purity of that chemical, the fit, the retrofit, the chemical formula and molecular weight. These are all indicators for chemical matches within the lists.

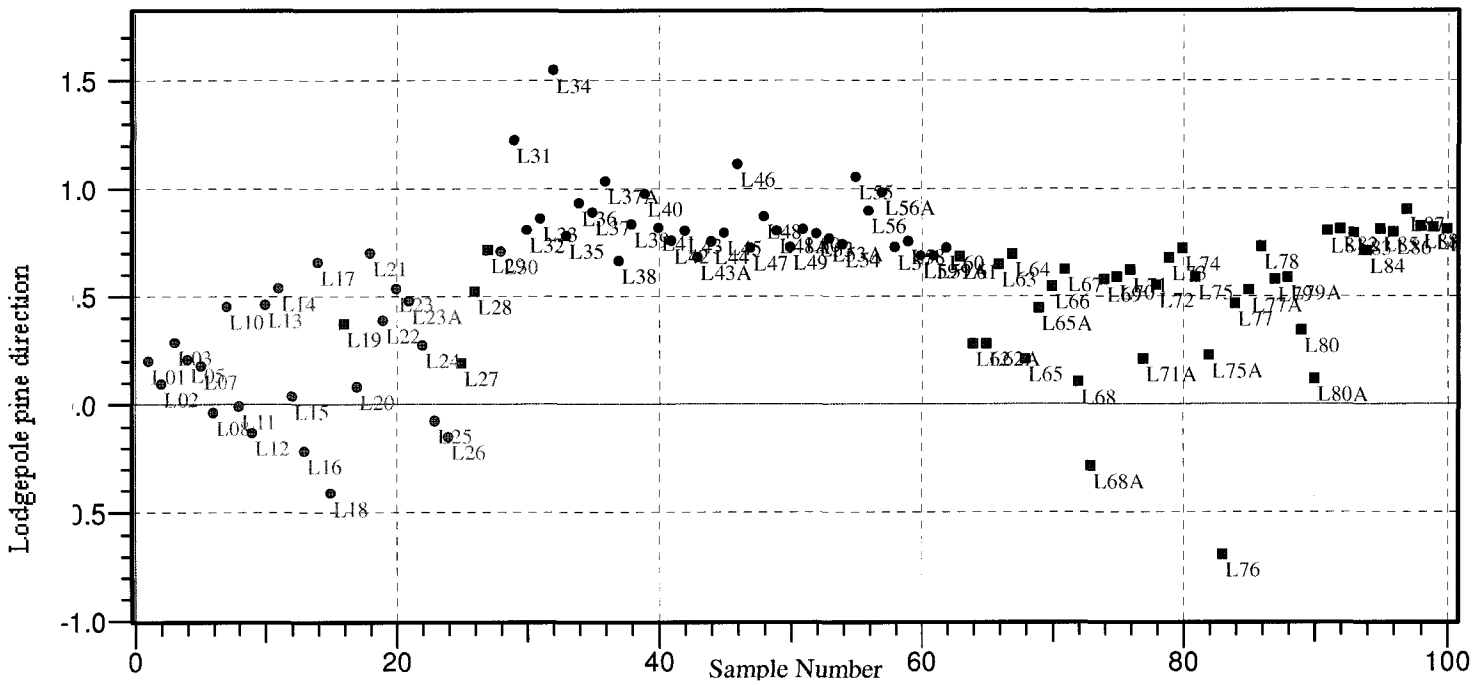


Figure 51: Sample groups based on the discriminant variable value calculated, and coordinating species group. The discriminant variable indicates how similar the samples are to lodgepole pine. Samples L01-30 = jack pine = sampling area, samples L31- 60 = lodgepole pine sampling area, samples L61-90 = hybrid sampling area.

Figure 51 shows variability between groups based on a prediction coefficient established from the PLS-DA. The prediction coefficient is used to determine how closely related each sample point is to lodgepole pine chemical characteristics. This figure demonstrates that there is a difference between lodgepole pine samples and the other species groups; groups show distance in coefficient value from lodgepole pine samples. Lodgepole and jack pine samples are represented by circles, while hybrid samples are represented by squares. Jack pine and hybrid samples fall mostly on the lower half of the graph with coefficients of 0.5 or less, while lodgepole pine samples fall mostly on the upper half of the graph with coefficient values greater than 0.5.

5.5.4 Statistical Analysis of Chromatographs

Table 23: Pairwise Comparison of Chromatographic peak regions with most variability between species groups. Species 1 = jack pine, species 2 = lodgepole pine, species 3 = hybrids, species 4 = hybrids from jack pine sampling area, species 5 = lodgepole pines from hybrid sampling area

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig. (a)	95% Confidence Interval for Difference(a)	
						Lower Bound	Upper Bound
Region5	1	2	789220.719(*)	194973.868	.000	402251.562	1176189.876
		3	760219.272(*)	200812.026	.000	361662.988	1158775.556
		4	934600.712(*)	399335.578	.021	142030.132	1727171.291
		5	1011188.762(*)	276667.804	.000	462079.756	1560297.767
	2	1	-789220.719(*)	194973.868	.000	-1176189.876	-402251.562
		3	-29001.447	189249.143	.879	-404608.610	346605.715
		4	145379.992	393647.903	.713	-635902.129	926662.114
		5	221968.042	268393.074	.410	-310717.914	754653.999
	3	1	-760219.272(*)	200812.026	.000	-1158775.556	-361662.988
		2	29001.447	189249.143	.879	-346605.715	404608.610
		4	174381.440	396571.976	.661	-612704.157	961467.036
		5	250969.490	272663.717	.360	-290192.512	792131.491
	4	1	-934600.712(*)	399335.578	.021	-1727171.291	-142030.132
		2	-145379.992	393647.903	.713	-926662.114	635902.129
		3	-174381.440	396571.976	.661	-961467.036	612704.157
		5	76588.050	439873.773	.862	-796439.626	949615.726
	5	1	-1011188.762(*)	276667.804	.000	-1560297.767	-462079.756
		2	-221968.042	268393.074	.410	-754653.999	310717.914
		3	-250969.490	272663.717	.360	-792131.491	290192.512
		4	-76588.050	439873.773	.862	-949615.726	796439.626
Region9	1	2	272772.100	206769.350	.190	-137607.824	683152.024
		3	796426.133(*)	212960.704	.000	373758.086	1219094.179
		4	904046.365(*)	423494.485	.035	63527.041	1744565.689
		5	846905.915(*)	293405.586	.005	264577.046	1429234.785
	2	1	-272772.100	206769.350	.190	-683152.024	137607.824
		3	523654.032(*)	200698.292	.011	125323.478	921984.587
		4	631274.265	417462.720	.134	-197273.674	1459822.204
		5	574133.815(*)	284630.253	.046	9221.552	1139046.079
	3	1	-796426.133(*)	212960.704	.000	-1219094.179	-373758.086
		2	-523654.032(*)	200698.292	.011	-921984.587	-125323.478
		4	107620.233	420563.692	.799	-727082.279	942322.744
		5	50479.783	289159.261	.862	-523421.307	624380.873
	4	1	-904046.365(*)	423494.485	.035	-1744565.689	-63527.041
		2	-631274.265	417462.720	.134	-1459822.204	197273.674
		3	-107620.233	420563.692	.799	-942322.744	727082.279
		5	-57140.450	466485.151	.903	-982984.345	868703.445

Dependent variable	(I) species	(J) species	Mean Difference (i-J)	Std. Error	Sig. (a)	Lower Bound CI 95% for difference (a)	Upper Bound Ci 95% for difference (a)
	5	1	-846905.915(*)	293405.586	.005	-1429234.78	-264577.046
		2	-574133.815(*)	284630.253	.046	-1139046.07	-9221.552
		3	-50479.783	289159.261	.862	-624380.873	523421.307
		4	57140.450	466485.151	.903	-868703.445	982984.345
		4	-53575.350	282297.045	.850	-613856.841	506706.141
Region11	1	2	496887.597	323529.351	.128	-145228.608	1139003.801
		3	1151234.281(*)	333216.882	.001	489891.009	1812577.553
		4	1298240.635	662636.390	.053	-16909.173	2613390.442
		5	1426385.385(*)	459087.958	.002	515222.870	2337547.899
	2	1	-496887.597	323529.351	.128	-1139003.801	145228.608
		3	654346.684(*)	314030.044	.040	31083.974	1277609.395
		4	801353.038	653198.564	.223	-495065.297	2097771.373
		5	929497.788(*)	445357.307	.040	45586.814	1813408.762
	3	1	-1151234.281(*)	333216.882	.001	-1812577.553	-489891.009
		2	-654346.684(*)	314030.044	.040	-1277609.395	-31083.974
		4	147006.353	658050.616	.824	-1159041.962	1453054.669
		5	275151.103	452443.788	.545	-622824.572	1173126.779
	4	1	-1298240.635	662636.390	.053	-2613390.442	16909.173
		2	-801353.038	653198.564	.223	-2097771.373	495065.297
		3	-147006.353	658050.616	.824	-1453054.669	1159041.962
		5	128144.750	729903.333	.861	-1320511.319	1576800.819
	5	1	-1426385.385(*)	459087.958	.002	-2337547.899	-515222.870
		2	-929497.788(*)	445357.307	.040	-1813408.762	-45586.814
		3	-275151.103	452443.788	.545	-1173126.779	622824.572
		4	-128144.750	729903.333	.861	-1576800.819	1320511.319

Region 5 indicates that jack pine is significantly different from lodgepole pine and hybrid samples. However, lodgepole pine is not significantly different from hybrid samples, perhaps indicating that the hybrids are more like lodgepole pine than jack pine in region 5. Species groups 4 and 5, which were unclear in species classification, show that they are only significantly different from jack pine.

Unlike region 5, region 9 displays no statistical difference between jack pine and lodgepole pine, but shows that there are significant differences between hybrids and pure species. Species group 4, made up of samples from the jack pine sampling region that show hybrid genetic output, show a significant difference from jack pine, but not other species groups. Species group 5, made up of samples from the hybrid area that show lodgepole pine genetic output, show a significant difference from both pure species but not from hybrids or species group 4.

Finally, region 11 shows that there was no significant difference between lodgepole pine and jack pine, but hybrids show significant difference from both pure species. Species group 4 show no significant difference from any group, and species group 5 show significant differences from both pure species, much like the hybrids.

5.6 Discussion and Conclusions

The primary objective of this chapter was to identify a "chemical extractive footprint" for the species groups and to determine whether or not this footprint could be used for differentiation between species. Variability between species ideally would take place in the chemical components of each species, however due to the similarities between these species groups variability was only established between quantities of the chemical compounds found.

Figures 47 through 49 encompass the analysis of variability between species groups according to chromatographic peak regions. The analysis reveals that not only are there some regions within the chromatograph (representative of certain chemical groups) that are found in unique quantities between the species groups, but the variation is sometimes statistically significant at $\alpha = 0.05$ according to the pairwise comparison conducted (Table 20).

Considering the results from all three regions together, hybrids can be considered significantly different from both jack pine and lodgepole pine according to the chemical variability of these regions. However, lodgepole pine and jack pine themselves can not be distinguished with an $\alpha = 0.05$ level of confidence. Species group 4 and 5 seem to have chemical characteristics more like hybrids than the pure species according to these chemical regions. Therefore, a chemical extractive footprint of distinguishing chemical quantities may be deduced for hybrid samples, but not for jack pine or lodgepole pine samples, according to the pairwise comparison.

To add to pairwise analysis, PLS-DA was preformed and Figure 47 was compiled in order to show how well each sample predicts a lodgepole pine chemical make up. As

shown, lodgepole pine samples can be separated from the others based on this predictive coefficient, indicating that hybrid samples may be more like jack pine samples than lodgepole pine samples according to chemical make up.

Showing variability between chemical make up among these species groups has been accomplished, however, several explanations exist to explain this variability. These differences are not necessarily due to a purely genetic component. Like other characteristics, age of sample, site conditions and growth rate, and genetics must all be considered. A significant increase in the amount of extractives present in sapwood is possible as the sapwood increases in age. There are also lower amounts of extractives in heartwood of young trees than older trees. In a study conducted on *Pinus echinata*, it was concluded that the age of the tree had more influence on extractive quantity than any other environmental variable (Higuchi, 1985). This is a major factor to consider in this study because the jack pine trees were younger than the lodgepole pine trees sampled, and the hybrid samples had more variation in age. With this in mind, and according to Higuchi (1985), it would be expected that lodgepole pine samples in this study would contain more extractives than jack pine samples. Fortunately age is not the only influence on extractive content, and variations on age influence were identified in peak region 5 and many others not investigated in detail, where jack pine was shown to have more extractives present than lodgepole pine.

Site conditions have been shown to affect the quantity of extractives present in some species, but no conclusive correlations have been made to show a direct relationship between growth rate and extractive content. The quantity of heartwood extractives is said to be negatively correlated with the rate of diameter growth (Higuchi,

1985). If this were an accurate trend, it would indicate that the jack pine samples in this study would have lower extractive contents than the lodgepole pine samples, due to a faster DBH growth rate. As concluded in examination of age difference, peak region 5 and many peak regions not investigated in detail show that jack pine has a greater quantity of extractives than lodgepole pine.

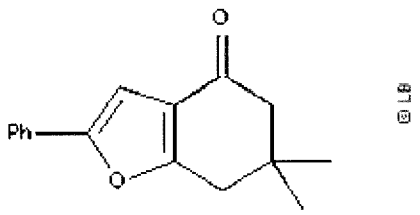
Figures 50a-f and the accompanying library search outputs, indicate the possible identification of the extractive compounds studied. In order to interpret the mass spectra and search output, it is important to factor in the molecular weight of the compounds, the retention time for the chemical from GC, the parent ion, the overall ion pattern, and the purity, and fit values given by the search.

All samples had the same mass spectral outputs for each region, indicating that the chemicals found in the samples were the same. Because the chromatographic peaks were different, the quantitative analysis is still valid even though the species groups share the same extractives in their makeup.

According to Fernandez et al. (2000), the chemical classes with GC retention times approximate to those found in this study, are fatty acids and monoglycerides. The retention time considered is from ~15.0 minutes to ~18.5 minutes (Figure 85). Based on relative retention times with the internal standard, heptadecanoic acid, retention times were matched to those found by Fernandez et al. (2000), in order to deduce possible chemical identities of the peaks analyzed by mass spectra in this study. The retention time of heptadecanoic acid in this study was 16.52 minutes. Peak region 5 contained two main peak areas, one at a retention time of 15.9 and one at 16.1 minutes; peak region 9 contained one main peak at a retention time of 17.7 minutes; and peak region 11

contained three peaks at retention times 18.3, 18.36, and 18.4 minutes. Relative retention times indicate that candidate chemicals for peaks in region 5 are o-coumaric acid, 4-hydroxy-2-methylacetopheone, and 9-oxononanoic acid. The peak in region 9 could be identified as eicosanoic acid according to Fernandez et al. (2000), and chemical candidates for peaks in region 11 include only possible broad chemical classes such as: sterol/triterpenes, diglycerides, or waxes.

Based on the output in Figures 50a-f and the library searches accompanying these figures, potential chemical identities or chemical classes can be suggested. Figure 50a, the first of three peaks in region 11, contains a potential match through library searches, this chemical was identified as a stilbene. This chemical compound has a molecular weight of 240 g/mol, which is the parent ion weight in the mass spectra. Stilbenes are naturally occurring phenolic constituents that originate from cinnamic acids and have specific chemical types that are commonly found in genus *Pinus* (Higuchi, 1985; Sjöström, 1993).

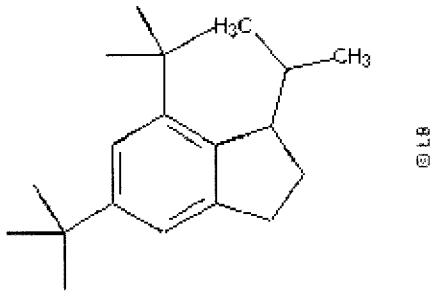


(Landolt Börnstein, 2004).

Figure 52a: An example of a compound similar to the stilbene identified as a candidate match.

The second and third peaks in region 11 (Figures 50b and c) have similar lists of potential chemicals according to their mass spectral output. Both are suggestive of a type of phenanthrene, which is related to bibenzyls and the stilbene family of compounds (Higuchi, 1985). This phenanthrene has a molecular weight of 272 g/mol which is

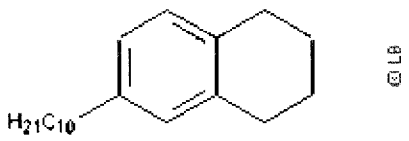
greater than the molecular weight of the parent ion and reflects a potential match to the library search.



(Landolt Börnstein, 2004).

Figure 52b: An example of a compound similar to the phenanthrene identified as a candidate match.

Peak region 9 had only one main peak in which to classify. In the library search, naphthalene was recognized as a potential chemical candidate. According to Higuchi (1985), naphthalenes are categorized as a type of quinone. Derivatives of this compound were found in several gymnosperms, so it is plausible that this compound is found in *Pinus*.



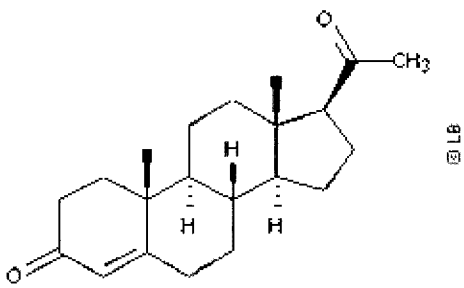
(Landolt Börnstein, 2004).

Figure 52c: An example of a compound similar to the naphthalene identified as a candidate match.

Lastly, peak region 5, containing two peaks of significance, was identified to provide variability between species groups in this study. Benzoic acid was identified as a potential candidate match to Figure 50e, and another phenanthrene is a potential match to Figure 50f. While the library match to benzoic acid was not the closest match, and does not show ideal numbers for purity or fit, it is a compound that was found to be present in the study by Fernandez et al. (2000), and is found at a relatively close retention time to

the chemical peak analyzed in this study. The molecular weight of benzoic acid is 254 g/mol which is acceptable for a potential match.

The phenanthrene candidate to Figure 50f is another stilbene (phenolic compound), but a different phenanthrene than the one identified in region 11. This phenanthrene has a molecular weight of 314 g/mol, which is also an acceptable match.



(Landolt Börnstein, 2004).

Figure 52d: An example of a compound similar to the phenanthrene identified as a candidate match.

Analysis on the mass spectrometry output for this study is only preliminary. Therefore conclusive identities of the compounds can not be deduced. A more detailed analysis of the mass spectra would allow for identification of species based on a chemical extractive footprint.

Another possible way to positive identify the compounds in question is to run pure samples of the candidate chemicals appearing in the library searches through the GC-MS, and then compare their outputs to the unknowns. A matching retention time would indicate a chemical match.

Improving the chromatographic warping due to stretch would also improve the accuracy of this study, and enable more information to be gathered from the chromatograms. According to Bylund et al. (2002), peak position and peak width often show variation, other than that caused by sample difference, due to output of a two

dimensional figure from a trilinear model. Alignment variation can be compensated for with slight peak adjustments as were made in this study. However, warping and stretch are more difficult to balance. Warping is caused by the age of the column used and flow-rate variation, therefore affecting the retention time of a chemical compound. Varied retention time for the same compound over several samples causes peaks that should be the same to appear skewed or stretched. Correlation optimized warping (COW) of chromatographic figures was designed to compress or expand peak profiles by linear interpolation to reduce variation in retention times (Byund et al., 2002). By applying COW to this study, more accurate representations of chemical profiles may be determined.

A preliminary assessment of chemical makeup of jack pine, lodgepole pine, and their hybrids has been provided in this chapter to act as further evidence of differentiation between species groups and compliment the findings in chapters 2, 3, and 4.

Quantitative variability does exist between jack pine, lodgepole pine, and hybrid samples. Preliminary analysis indicates that wood extractives may place the samples in this study more like jack pine than lodgepole pine. However, chemical quantities thus far do not demonstrate the intermediacy theory in hybrids to an extent that would be statistically significant. It is possible that site conditions, age of samples, and genetic heritability all played a role in the established extractive content of the samples examined; due to which a clear "chemical extractive footprint" can not be determined. Further manipulation of chromatographs and more extensive investigation of mass spectra are required.

5.7 References

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Chapter 6: Discussion of and Applications for Hybridization of Lodgepole and Jack Pine

6.1 Hybridization

6.1.1 Predictive Abilities of Characteristics

Chloroplast DNA from the *mat*k gene and mitochondrial DNA from the *nad*1 gene both successfully identified parental lineage of the lodgepole pine, jack pine, and hybrid samples in this study.

Using knowledge of the predominant species for each sampling area as well as the genetic information gathered using cpDNA and mtDNA, a comparison of species groups based on other morphological, cell and fibre, and chemical characteristics was attempted. This comparison was preformed in Chapters 3, 4, and 5 in order to determine if any of these characteristics are capable of distinguishing between species, or between hybrids and species. It was also interesting to determine if any of the characteristics placed hybrid samples as intermediate of the two pure species, thus illustrating what is happening genetically, the combination of genetic material 50% from jack pine, and 50% from lodgepole pine. Morphologically, needle pair size represented as a ratio of needle V width/needle length, and cone angle of attachment were the most useful characteristics for differentiating between all species groups (including the distinction of hybrids) while also demonstrating intermediacy in hybrids. Cone length had the ability to distinguish hybrids from pure species, but no discriminatory abilities were noted for differentiation between jack pine and lodgepole pine. Cone length did not demonstrate intermediacy.

Wood and fibre traits revealed that modulus of elasticity was the most useful characteristic for discriminating between species groups, and also showed hybrid samples to be intermediate. Cell wall thickness and fibre coarseness distinguish pure species from

each other, and distinguishes between hybrids and lodgepole pine, but not between hybrids and jack pine, suggesting that these two latter species may be more similar based on these two characteristics. Cell wall thickness and fibre coarseness do not demonstrate intermediacy. Microfibril angle was useful in this study for distinguishing between pure species but not separating out hybrid samples. Lastly, moisture content, DBH growth rate, and earlywood/latewood contents were seen to distinguish between species groups but are subjective in their interpretation due to their strong relationship with site conditions.

Chemical analysis via GC-MS revealed that peak regions 5, 9, and 11 have characteristic quantities of chemicals for pure species and hybrids, therefore distinguishing hybrids from pure species groups. PLS-DA suggested that chemical makeup of hybrids may be more like that of jack pine than lodgepole pine. No intermediacy of hybrids was shown through chemical analysis.

Since the characteristics mentioned above possess the ability to distinguish between species groups, it is possible to use them as predictive tools for hybridization. If a certain characteristic is measured, and falls within the measurement range that is characteristic for a hybrid as opposed to a pure jack pine or lodgepole pine, then assumptions can be derived that the species is a hybrid without having to conduct any genetic analysis. This information can then be used by managers or manufacturers to alter their practices to best suite the material they are working with. Managers may adjust growing conditions to account for trees with faster or slower growth rates, and manufacturers may alter production to account for varying MOE or latewood content in raw wood. Wood that is

being used for pulp products may be sorted according to variation in fibre coarseness or quantity of chemical extractives in order to be processed more accurately.

Hybridization is not only important to distinguish from a production point of view, but also from a scientific point of view. Hybrids occur naturally between many different organisms, and cause controversy over the definition of a species. A species is defined as a group of organisms that interbreed but are reproductively isolated from other populations (Barton and Hewitt, 1985). Since hybrids are the product of two supposedly reproductively isolated populations, it is not certain whether hybrids should be classified as a new species, or if original taxonomic classification should be considered inaccurate. Taxonomists and other researchers can use the information in this study to assign characteristic limitations to new species and subspecies groups being developed.

6.1.2 Hypotheses and Zoning

Two hypotheses exist regarding the presence of a hybrid introgression zone between jack pine and lodgepole pine in Alberta and Northern BC. Hybridization; the crossing of genetic information between species, occurring where the two pure species ranges overlap, is the first hypothesis. The alternate suggests that jack pine and lodgepole pine diverged from one species into the two species that exist today. Understanding hybrid zones and species movement helps to better define which hypothesis may be more accurate.

Woodruff (1973) classified hybrid zones according to the pattern of species distribution (Figure 1). Allopatric zones feature natural hybrids that occupy a narrow area between pure parental species; sympatric zones feature hybrids and parental species in the same areas. Sympatric zones can be broken down further into parapatric zones;

parental species remain localized (segregated) while hybrids integrate with both parental species at their borders. Parapatric hybridization is the closest model to describe the pattern of species distribution among jack pine, lodgepole pine, and their hybrids. It has been suggested by Barton and Hewitt (1985) that allopatric zones indicate well-equilibrated populations, while sympatric populations indicate populations that are still exchanging large amounts of genetic information. This is directly related to the length time these populations have been hybridizing; stability in populations is said to be achieved over time. Applying this to parapatric populations in this study suggests that genetic information is still being exchanged between study groups and equilibrium has not been reached. This may also mean that these populations have only been hybridizing for a relatively short period of evolutionary time.

Opposing the hypothesis of hybridization between jack pine and lodgepole pine exists the hypothesis of species divergence. Divergence implies that over evolutionary time, jack pine and lodgepole pine were produced from one species, and have been continually evolving into two species due to environmental influences. This explains the presence of some trees that exhibit characteristics and genetic information from both jack pine and lodgepole pine, however, hybridization may better explain this finding.

Based on the evolutionary time required to diverge a species into two separate species, with only a cline of hybrids left as a record of the original, it is improbable that this divergence took place. Barton and Hewitt (1985) suggests that divergence of two populations could not take place without a "...virtually complete barrier..."; the Rocky Mountains could be seen as such a barrier, however, pines do cover this expanse, and a larger physical barrier does not exist. Hybridization or divergence could only have taken

place since the last ice age. In order to have almost completely diverged, with no solid genotype or phenotype of a predecessor species left, these pines must have been evolving very quickly. Hybridization however, could have begun after the last ice age, as jack pine and lodgepole pine ranges grew closer together, and therefore created the cline of phenotypes that are in existence today. Lastly, Danick and Yeh (1983) calculated the genetic distance of jack pine and lodgepole pine to be much greater than genetic distances within species, supporting the differentiation between these two pines. If divergence has occurred over time, it might be expected that the genetic distance between species would be more similar to within species variation, or smaller than other between species distances.

These hypotheses cannot be verified without a close inspection of the fossil record, and in depth genetic analysis of multiple hybrid populations to determine genotype relationships. Even then it may be impossible to say if one hypothesis is more valid than another. Based on geographic range alone, it is shown that jack pine and lodgepole pine having been migrating towards one another, now virtually undivided rather than migrating away from one another into their own environments over time.

6.1.3 Maternity and Paternity Issues: Effects on Productivity

Maternal and paternal genotypes contribute directly to characteristics such as germination percentage, rate of growth, and fitness of the tree even though environmental conditions also play a role. Productivity of a mature tree can be linked to the genetic information contained within the endosperm, or nutritive tissue, of the seed and within the embryo. The endosperm is formed from only maternal genes, and regulates the initial growth rate of the germinant. The embryo is composed of both maternal and paternal

genetic information. These genotypes determine growth rates which affect the germinant's ability to compete for nutrients and light. The fittest trees will out compete other seedlings and become stronger in later stages, producing trees with greater volume at maturity up to a certain age (Fries et. al., n.d.). This supports the idea that individual trees originating from a hearty, more evolve maternal line have an increased chance of producing higher volumes of quality wood because of an initial growth advantage. Therefore, strong, hearty trees that are involved in hybridization will undoubtedly produce seedlings with these genotypes that can better compete for survival.

Hybrids are produced by evolutionary processes, and recombination of genetic information allows for improved survival for select populations. These populations go on to produce Fn generations of individuals with improved tolerance of various environments as well as a variety of other adaptations to combat pests, pathogens, or other factors that may threaten survival of the species. In a natural environment, this process takes thousands if not millions of years to significantly change a population. However, today, lab controlled crosses can mimic or interpret the advancement of a species in order to produce populations of hybrids that possess an array of characteristics for various purposes. Hybrids can provide improved growth rate, improved frost tolerance for cold climate plantations or general northern environments, improved wood quality, improved crown shape and size, or improved pest or pathogen resistance (Savolainen and Kärkkäinen, 2004). Hybrid vigor is not displayed by intermediate triats, as were seen in some of the hybrid characteristics identified in Chapters 3 and 4. Therefore, although intermediacy in characteristics is more effective for hybrid identification, it is not always desirable for traits that may provide increases in yield,

quality, or tolerance when managing for anthropological use. For example, intermediate MOE is not as desirable as hybrids that demonstrate higher MOE for dimensional lumber manufacturing.

Natural hybrids can provide seed sources with already evolved genetic material. The lodgepole pine x jack pine hybrids that exist in the Northeast corner of British Columbia, exhibit characteristics varying from the parental species, and may therefore offer increased value to some products, depending on what traits are being sought. These trees may have a greater potential for frost resistance than other pines produced due to the latitude at which they were developed. Frost resistance is an important trait to consider when developing a plantation. Trees that are damaged by frost multiple times become shrub-like with no vertical growth and very little secondary growth. These populations are of low quality and therefore low value due to excessive stem deformation, branchiness, and lack of wood volume. Results from a study conducted in Sweden revealed that traveling 1° in latitude northward (in the northern hemisphere) caused a 10 % decrease in survival rate for Scots pine (*Pinus sylvestris*). Similarly, increases of 100m in elevation lead to a 3% reduction in survival (Savolainen and Kärkkäinen, 2004). For these reasons, it is pertinent for forest managers to select the best seed possible for reforesting more northern regions of the world, such as the areas sampled for this study.

One down fall to production of hybrid crosses, both naturally and artificially, is their reproductive capabilities. According to Critchfield (1985), lodgepole pine and jack pine can be very easily crossed in a laboratory setting. Crosses that are performed with lodgepole pine as the female parent result in approximately 30% germinal seeds, which is

very low. Also, putative hybrids in Alberta have been observed to produce aborted pollen grains or pollen grains that are smaller than non-hybrids. Forty-two percent of putative hybrids have aborted pollen as compared to only 1-2% in pure species, making hybrid proliferation difficult (Critchfield, 1985).

6.2 Applications

6.2.1 Hybridization Effects on Management for Wood Quality

Information regarding the specific chemistry, wood fibre properties, and growth of hybrids can be applied to management of cold climate plantations to ensure optimum yield. Hybrids have the potential to grow faster than predecessor species as they are an evolved combination of the two parents. Management practices can be altered to accommodate more specific needs of each species cross rather than assuming lodgepole pine or jack pine criteria. For instance matching an area with the most adapted variation of pine hybrid when reforesting, to ensure maximum growth and eventually yield.

Hybrids will be heartier, faster growing, better at adapting to environmental conditions, and possibly even more resistant to pathogens and pests than pure species are. Evolving hybrids of two pre-existing species will allow the tree to take on the most advantageous characteristics of the predecessors.

Wood that is more uniform is generally considered better quality. Uniformity, meaning the absence of knotting, scarring, or reaction wood, and a consistent pattern of earlywood versus latewood rings, can be improved with management techniques. Silvicultural treatments such as site preparation and fertilization allow seedlings and immature trees to allocate energy towards stem growth rather than defense mechanisms or root expansion. Fertilizers have generally been used to increase growth rates, and site

preparation is generally used to eliminate competitive species and improve soil drainage. In pines such as jack and lodgepole pine, wood produced from nitrogen fertilization results in short cells with thin walls and therefore lower density (Zobel, 2004).

Other silvicultural techniques that can be applied to change wood quality for specific products include spacing and pruning. Varying stand density by spacing/thinning changes the ring widths (Zobel, 2004) and EW:LW ratios, and therefore affects manufacturing and processing because ring width is directly associated with wood density and wood uniformity. Pruning stands eliminates lower branches, forcing the tree to allocate energy towards stem growth rather than branch growth, resulting in higher quality, larger stems for solid wood production (Zobel, 2004). Pruning is not as common in pine stands, because generally pines will self-prune after a certain age.

Wood quality is said to be strongly inherited and therefore using genetically improved tree stock through methods such as hybridization and selective breeding, in combination with silvicultural management techniques, has potential to greatly improve wood uniformity as well as other wood characteristics (van Buijtenen, 2004). Operationally, several methods can be used to genetically manipulate seed stock for improved wood quality traits. Using clonal forestry or seed orchards are the most common approaches. Clonal forestry uses vegetative propagation to reforest areas using root cuttings or other tissue cultures from one or two parents. This method can be expensive, so often other methods are sought (van Buijtenen, 2004) however clonal populations have been used very successfully for low costs with Eucalyptus hybrids (Potts, 2004). One method that exists for clonal regeneration of improved seedlings is somatic embryogenesis. This method involves utilization of asexual clone embryos from

a single seed source. Embryos are grown on culture plates and then transferred into small seedling containers. These seedlings are genetically identical and have been used for successful spruce plantations, and more recently investigated pine plantations (Klimaszewska, 2005). This method is successful in plantation based areas but is not implemented in natural reforested areas such as forests in British Columbia for key reasons. These include the limitations that clonal forestry poses on biodiversity, and risks associated with reforesting large areas of land with only one or two genotypes, such as pest or pathogen infestation, or poor resistance to elements such as drought or frost.

Seed orchards also utilize one or two parents with the most desired qualities, but graft these individuals in an orchard. Orchards are then inspected, and trees displaying undesirable traits are discarded. Seed produced by the remaining orchard trees is used for operational needs (van Buijtenen, 2004).

Learning from naturally hybridized forest stands such as the jack pine x lodgepole pine stands investigated in this study will enable the scientific community to better understand what these populations have to offer the forest industry, and how they should be managed for various purposes. Since these natural populations have been growing and evolving over a long period of time, they lend insight into affects that hybridization may have on environments and wood quality that are not obvious from newly formed plantation based progeny.

6.2.2 Hybridization effects on Manufacturing and Processing

Wood fibre properties looked at determined if hybrids were of better quality for manufacture and processing of wood or pulp than lodgepole or jack pine for certain products. Knowledge of fibre properties of the hybrids is useful for processing.

Manufacturing and processing could be optimized for specific hybrid characteristics of the wood, rather than using generic settings and assumptions of pure species to manipulate the boards or fibres.

Equipment and methods for processing and manufacturing wood products is often altered in order to more efficiently utilize various types of wood. With this increased efficiency however, costs can increase or the quality of the final products can be compromised. Production becomes stable and effective when raw materials are relatively uniform (Zobel, 2004). It may be possible to find and manipulate populations or closely related species by mimicking natural hybridization as seen in jack pine and lodgepole pine, in order to produce a hybrid that is genetically improved compared to its predecessors. If this is possible, this manipulation could lead to more uniform wood for processing and manufacturing of wood products.

Wood quality needs vary depending on what is being produced. For example, a low density stem may be considered less valuable than a higher density stem, however, if the desired product is writing paper or tissue, than low density is actually preferred. Higher density wood is better for fibre boards or increased pulp yields (Zobel, 2004). All of the other wood and fibre characteristics investigated in this study have various impacts on the quality of the end product. Low microfibril angles are important for product stability in solid wood products and quality of paper products, and thin-walled cells as well as large lumens are important for production of tissue paper (Zobel, 2004). Hybridization of species and populations offers evolved variations in wood, allowing for differences in wood characteristics, which may improve quality for specific products.

Wood characteristics under genetic control, as confirmed to date, include latewood percentage, cell dimensions, chemical properties, and microfibril angle. Other traits are being investigated for their genetic applicability. Latewood percentage ranges in heritability depending on the population in question, however little more is known about EW:LW genetic relationships. Tracheid length, diameter, and cell wall thickness are all strongly inherited; variation in populations has been noted in lodgepole pine. Chemical properties have been studied in relation to genetic inheritance since the 1970's, and genetic improvements in these areas have been accomplished. Lignin content has been studied most extensively because of its applications in the paper making process, while less is known about genetic improvements to cellulose content. Lastly, very little is known about microfibril angle heritability, this trait is still under investigation (van Buijtenen, 2004).

Knowledge of natural hybridization could potentially lead to management for select traits in order to obtain the best wood quality output for certain products and for optimal processing and manufacturing. This could potentially reduce waste wood, increase yield, and allow for more cost effective production.

6.3 References

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Appendices:

Appendix 1:
Site maps

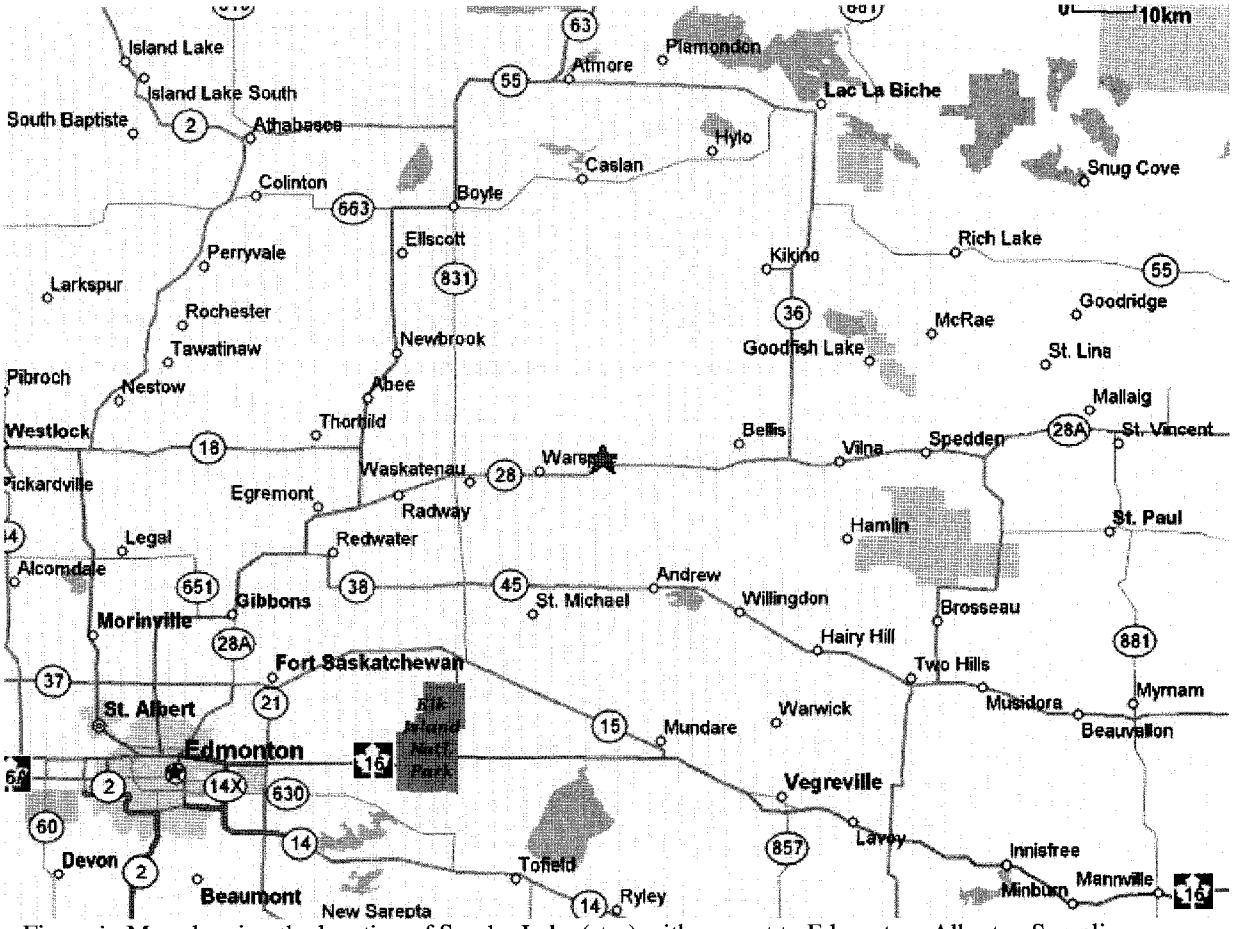

Figure i: Map showing the location of Smoky Lake (star) with respect to Edmonton, Alberta. Sampling sites fall east of Smoky Lake, towards Bellis (MapQuest, 2005).

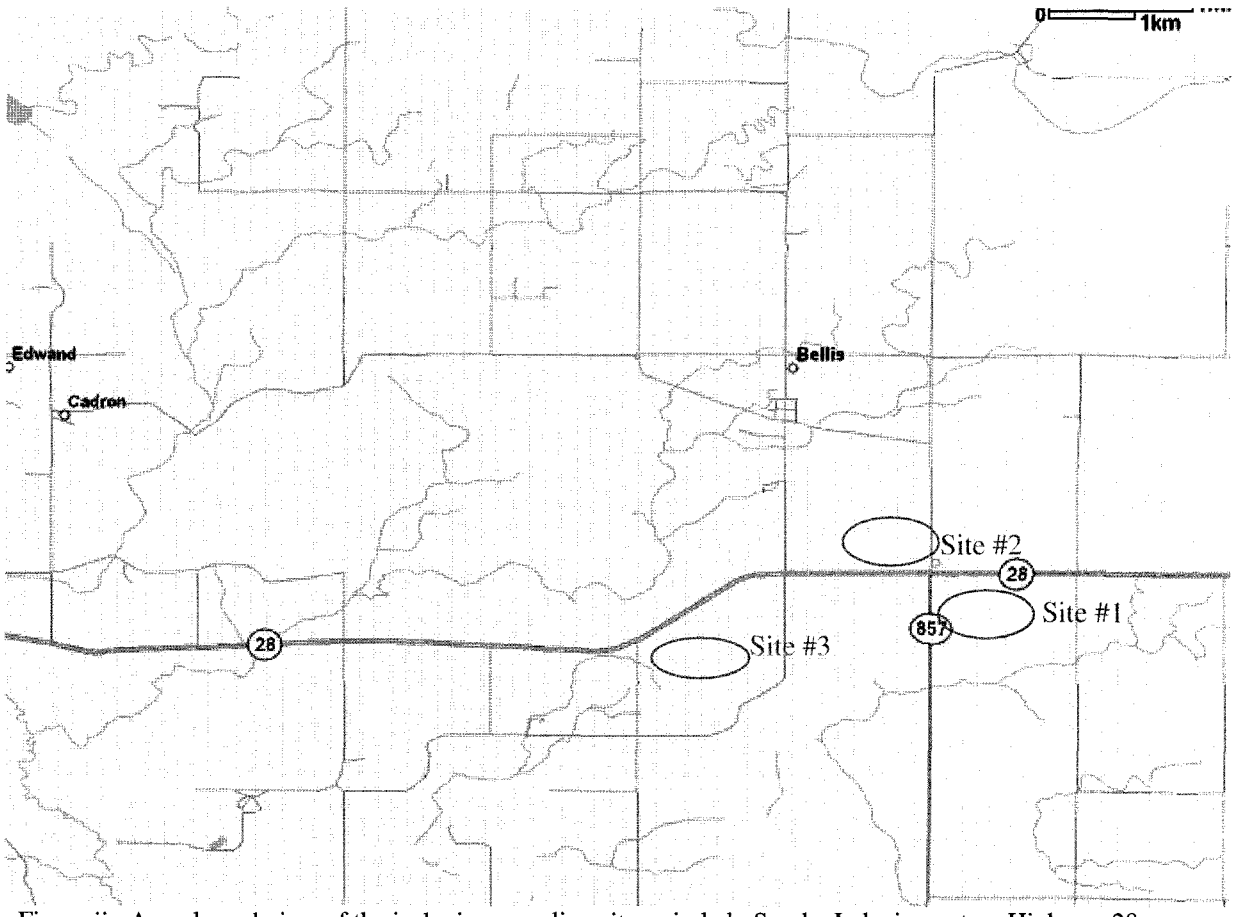

Figure ii: An enlarged view of the jack pine sampling sites, circled. Smoky Lake is west on Highway 28 (MapQuest, 2005).

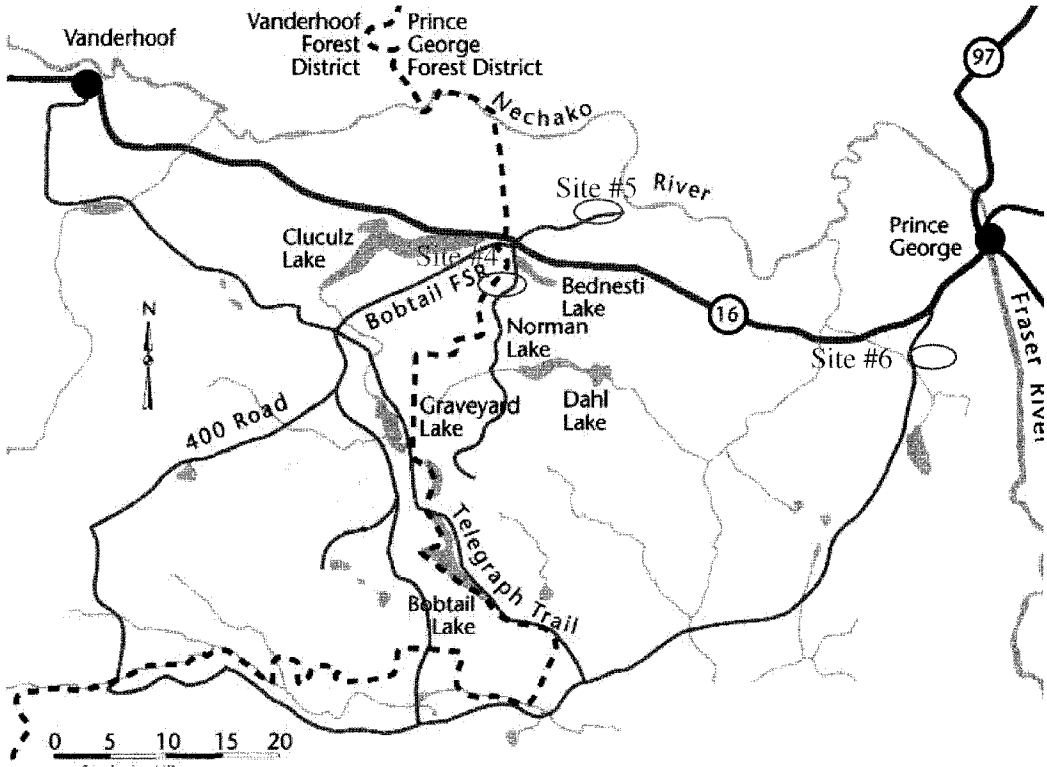

Figure iii: Map of Bobtail FSR and Gregg Creek FSR west of Prince George. Sites 4, 5, and 6 are circled (Sanborn et. al., 2001).

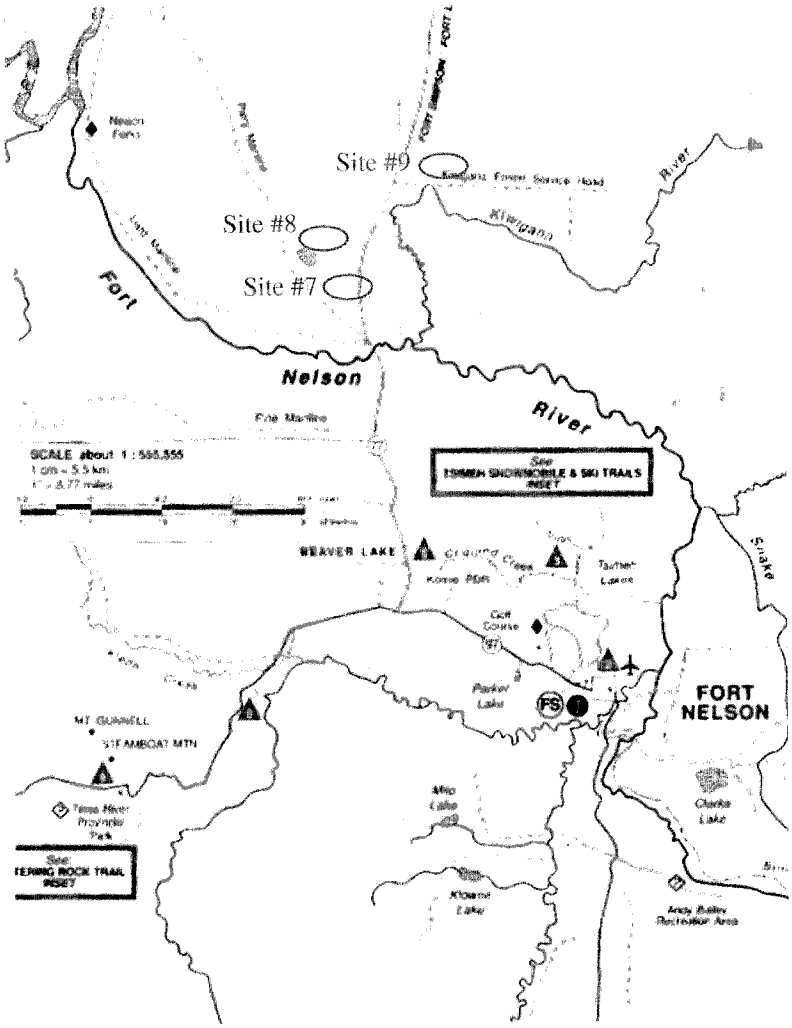

Figure iv: Site map for Fort Nelson area sampling sites. Sites are circled.

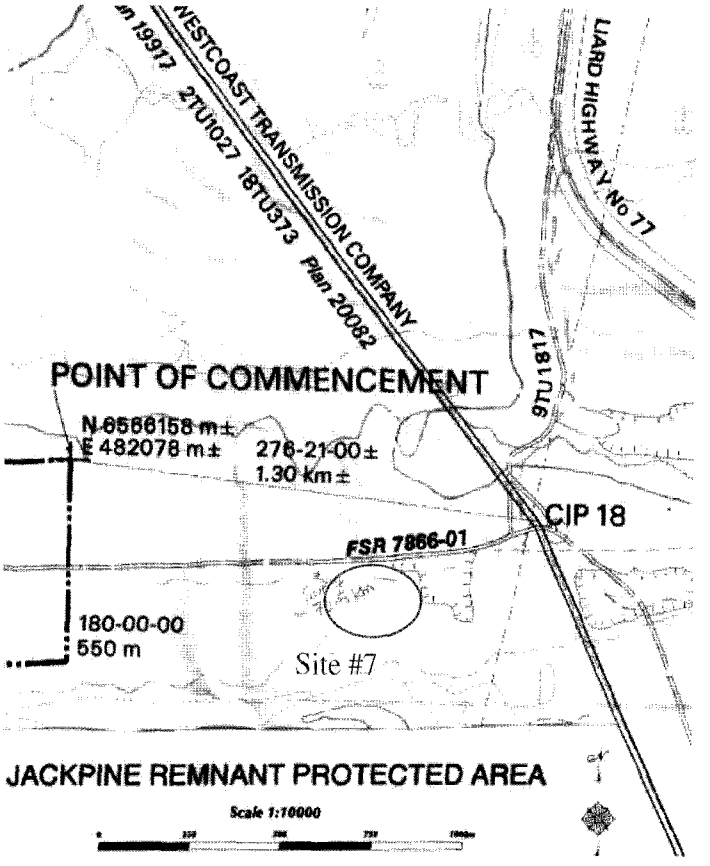

Figure v: An enlarged and more detailed view of the site 7 location. Forest Service Road (FSR) 7866-01 is the Patry Mainline.

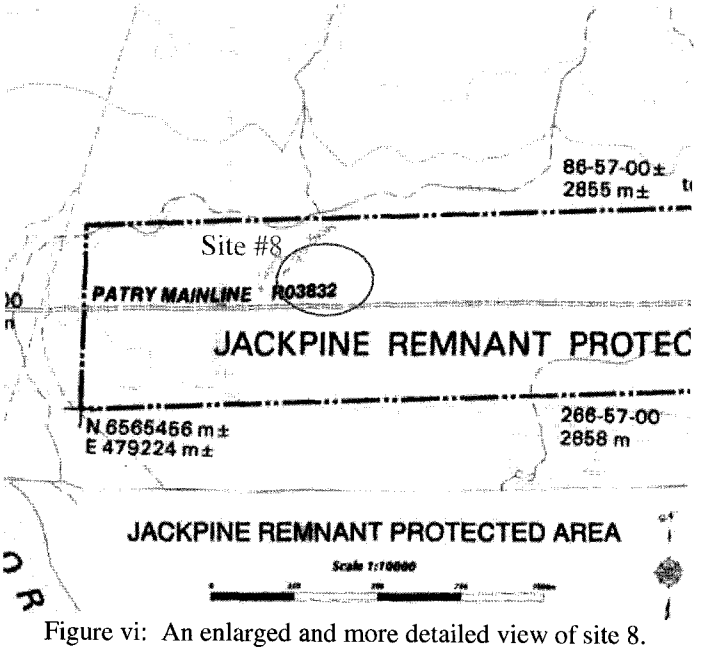

Figure vi: An enlarged and more detailed view of site 8.

References:

MapQuest® Inc. 2005. www.mapquest.com Accessed November 6, 2005.

Sanborn, Paul, Rob Brockley, and Caroline Preston. 2001. Effects of Sitka Alder Retention and Removal on the Growth of Young Lodgepole Pine in the Central Interior of British Columbia. Establishment Report, EP 1185. Ministry of Forests Research Program, British Columbia.

Appendix 2:

Genetic Sequences for CpDNA matk gene markers in *Pinus banksiana* (restriction sites are underlined, primers are italicized):

i. CpDNA Section 1

```
1    atggatgagt tccatagatg cggaaaggaa gatagctttt ggcaacaatg ctttttatat
61   ccactctttt ttaaggaaga tctttacgca atttctcatg atcattattt ggatgtatca
121  agttcctcca gaccgatgga acatttaagt tccaatgatc aattaagttt cctaactgta
181  aaacgtttga ttggtcaaat acgtaaacaa aatcattcaa ttgttttatt cgygaattgc
241  gatccaaatc cattagctga tcgcaagaag agtttctatt ctgaatcggt actagaagca
301  cttacattgg tcctggaagt tccgttctct atatggtcaa aatattctgt ggaagggatg
361  aatgaatcga agagtttccg gtcgatccat tcaatatttc ccttcttaga ggataaattc
421  ccgcattcaa attctatatt agatgcacga ataccctatt ctattcatcc ggaaattttg
481  gttcgaacct ttcgtcgctg gatccgagat gctccctcct tgcacccatt acgatctgtt
```

ii. CpDNA Section 2

```
1081                                                        attctgtgac
1141 atatcagggc ggccaattag taaattgtct tggaccagtc taacagatga tgatatcctc
1201 gatcgattcg atcaaatttg gagaaatctt tttcattact acagtggatc ctttgatcga
1261 gatggtttat atcgtataaa gtatatactt tcattatcat gtgctaaaac tttagcctgt
1321 aaacataaaa gtacgatacg tgtagttcgg aaggaattag gtccagaact ctttaaaaaa
1381 tcgttttcaa aagaacgaga atttgattct ctgcgctttt catcaaaagc ggcggcccgt
1441 tcgcagagag aacgaatttg gcattcagat attccccaga taaatcccct agctaattcc
1501 tggcaaaaga tacaggatct taaaatagaa aacttatttg accaatgaaa tgctctttga
1561 gtaattgcct cgattcagaa tcatttttat ttttctatcc gagaactaaa atgattagga
1621 aatagataca ttacatgggg aaagccgtgt gcaatgagaa
```

Table i: Haplotype chart for determining the maternity of samples based on RFLP categorization from digestions with *Rsa*1, *Mbo*1, and *Hha*1 using the mtDNA nad1 gene.

sample #	species area	*Rsa*1	*Mbo*1	*Hha*1	Maternity by haplotype
2	Pj	A	A	A	Pj
5	Pj	A	A	A	Pj
8	Pj	A	A	A	Pj
9	Pj	A	A	A	Pj
13	Pj	A	A	A	Pj
14	Pj	A	A	A	Pj
15	Pj	A	A	A	Pj
16	Pj	A	A	A	Pj
18	Pj	A	A	A	Pj
19	Pj	B	B	B	Pli
20	Pj	A	A	A	Pj
21	Pj	A	A	A	Pj
22	Pj	A	A	A	Pj
23	Pj	A	A	A	Pj
24	Pj	A	A	A	Pj

sample #	species area	*Rsa*1	*Mbo*1	*Hha*1	Maternity by haplotype
25	Pj	A	A	A	Pj
26	Pj	A	A	A	Pj
27	Pj	B	B	B	Pli
28	Pj	B	B	B	Pli
29	Pj	B	B	B	Pli
30	Pj	A	A	A	Pj
31	Pli	B	B	B	Pli
32	Pli	B	B	B	Pli
35	Pli	B	B	B	Pli
36	Pli	B	B	B	Pli
37	Pli	B	B	B	Pli
38	Pli	B	B	B	Pli
39	Pli	B	B	B	Pli
40	Pli	B	B	B	Pli
41	Pli	B	B	B	Pli
42	Pli	B	B	B	Pli
43	Pli	B	B	B	Pli
44	Pli	B	B	B	Pli
45	Pli	B	B	B	Pli
46	Pli	B	B	B	Pli
47	Pli	B	B	B	Pli
48	Pli	B	B	B	Pli
49	Pli	B	B	B	Pli
50	Pli	B	B	B	Pli
51	Pli	B	B	B	Pli
52	Pli	B	B	B	Pli
53	Pli	B	B	B	Pli
54	Pli	B	B	B	Pli
55	Pli	B	B	B	Pli
56	Pli	B	B	B	Pli
57	Pli	B	B	B	Pli
58	Pli	B	B	B	Pli
59	Pli	B	B	B	Pli
60	Pli	B	B	B	Pli
61	Px	B	B	B	Pli
62	Px	B	B	B	Pli
65	Px	B	B	B	Pli
66	Px	B	B	B	Pli
67	Px	B	B	B	Pli
68	Px	B	B	B	Pli
69	Px	B	B	B	Pli
70	Px	B	B	B	Pli
71	Px	B	B	B	Pli
72	Px	B	B	B	Pli
73	Px	B	B	B	Pli
75	Px	B	B	B	Pli
77	Px	B	B	B	Pli
78	Px	B	B	B	Pli

sample #	species area	*Rsa*1	*Mbo*1	*Hha*1	Maternity by haplotype
79	Px	B	B	B	Pli
80	Px	B	B	B	Pli
81	Px	B	B	B	Pli
83	Px	B	B	B	Pli
85	Px	B	B	B	Pli
86	Px	B	B	B	Pli
87	Px	B	B	B	Pli
88	Px	B	B	B	Pli
89	Px	B	B	B	Pli
90	Px	B	B	B	Pli

Table ii: Haplotype chart for determining the paternity of samples based on RFLP categorization from digestions with *Snab*1 and *Hha*1 using the cpDNA matk gene.

sample #	species area	*Snab*1	*Hha*1	paternity by haplotype
2	Pj	A	A	Pj
5	Pj	A	A	Pj
8	Pj	A	A	Pj
9	Pj	A	A	Pj
13	Pj	B	A	Pj
14	Pj	B	A	Pj
15	Pj	A	A	Pj
16	Pj	A	A	Pj
18	Pj	A	A	Pj
19	Pj	B	A	Pj
20	Pj	A	A	Pj
21	Pj	C	A	Pj
22	Pj	C	A	Pj
23	Pj	C	A	Pj
24	Pj	A	A	Pj
25	Pj	A	A	Pj
26	Pj	C	A	Pj
27	Pj	A	A	Pj
28	Pj	A	A	Pj
29	Pj	A	A	Pj
30	Pj	A	A	Pj
31	Pli	D	B	Pli
32	Pli	D	B	Pli
35	Pli	D	B	Pli
36	Pli	D	B	Pli
37	Pli	D	B	Pli
38	Pli	D	B	Pli
39	Pli	D	B	Pli

sample #	species area	*Snab*1	*Hha*1	paternity by haplotype
40	Pli	D	B	Pli
41	Pli	D	B	Pli
42	Pli	D	B	Pli
43	Pli	D	B	Pli
44	Pli	D	B	Pli
45	Pli	D	B	Pli
46	Pli	D	B	Pli
47	Pli	D	B	Pli
48	Pli	D	B	Pli
49	Pli	D	B	Pli
50	Pli	D	B	Pli
51	Pli	D	B	Pli
52	Pli	D	B	Pli
53	Pli	D	B	Pli
54	Pli	D	B	Pli
55	Pli	D	B	Pli
56	Pli	D	B	Pli
57	Pli	D	B	Pli
58	Pli	D	B	Pli
59	Pli	D	B	Pli
60	Pli	D	B	Pli
61	Px	A	A	Pj
62	Px	A	A	Pj
65	Px	A	A	Pj
66	Px	A	A	Pj
67	Px	A	A	Pj
68	Px	A	A	Pj
69	Px	A	A	Pj
70	Px	A	A	Pj
71	Px	A	A	Pj
72	Px	A	A	Pj
73	Px	A	A	Pj
75	Px	A	A	Pj
77	Px	A	A	Pj
78	Px	A	A	Pj
79	Px	A	A	Pj
80	Px	A	A	Pj
81	Px	D	B	Pli
83	Px	D	B	Pli
85	Px	D	B	Pli
86	Px	D	B	Pli
87	Px	D	B	Pli
88	Px	D	B	Pli
89	Px	D	B	Pli
90	Px	D	B	Pli

Appendix 3: Statistical Output

Morphology:

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	species	Mean	Std. Deviation	N
needle_w_l	1	.487527	.1258983	25
	2	.168409	.0895083	18
	3	.406732	.1083569	18
	4	.481770	.0850018	4
	5	.222111	.0794389	10
	Total	.355852	.1704308	75
cone length (mm)	1	38.326	6.5674	25
	2	35.256	5.0294	18
	3	41.757	6.1080	18
	4	29.646	.9869	4
	5	41.238	4.2827	10
	Total	38.338	6.4043	75
Height/Age	1	.287649	.0422417	25
	2	.292295	.0465218	18
	3	.270460	.0724188	18
	4	.315020	.0487452	4
	5	.267249	.0296795	10
	Total	.283378	.0513766	75
DBH/Age	1	.485633	.1020937	25
	2	.299126	.0645270	18
	3	.332194	.0787029	18
	4	.446961	.0713601	4
	5	.415617	.0651152	10
	Total	.392648	.1124738	75
cone angle	1	-4.630075	9.8775189	25
	2	56.638702	6.5662406	18
	3	40.246528	20.1071602	18
	4	-12.260290	15.5378457	4
	5	60.049534	19.6343089	10
	Total	29.061819	31.8129985	75

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	needle_w_l	1.355(a)	4	.339	29.834	.000
	cone length (mm)	767.783(b)	4	191.946	5.926	.000
	Height/Age	.011(c)	4	.003	1.095	.366
	DBH/Age	.456(d)	4	.114	16.653	.000
	cone angle	60751.523(e)	4	15187.881	75.180	.000
Intercept	needle_w_l	6.228	1	6.228	548.559	.000
	cone length (mm)	69203.916	1	69203.916	2136.517	.000
	Height/Age	4.096	1	4.096	1559.704	.000
	DBH/Age	7.820	1	7.820	1141.145	.000
	cone angle	39137.894	1	39137.894	193.732	.000
species	needle_w_l	1.355	4	.339	29.834	.000
	cone length (mm)	767.783	4	191.946	5.926	.000
	Height/Age	.011	4	.003	1.095	.366
	DBH/Age	.456	4	.114	16.653	.000
	cone angle	60751.523	4	15187.881	75.180	.000
Error	needle_w_l	.795	70	.011		
	cone length (mm)	2267.370	70	32.391		
	Height/Age	.184	70	.003		
	DBH/Age	.480	70	.007		
	cone angle	14141.426	70	202.020		
Total	needle_w_l	11.647	75			
	cone length (mm)	113270.260	75			
	Height/Age	6.218	75			
	DBH/Age	12.499	75			
	cone angle	138237.150	75			
Corrected Total	needle_w_l	2.149	74			
	cone length (mm)	3035.153	74			
	Height/Age	.195	74			
	DBH/Age	.936	74			
	cone angle	74892.949	74			

a R Squared = .630 (Adjusted R Squared = .609)
b R Squared = .253 (Adjusted R Squared = .210)
c R Squared = .059 (Adjusted R Squared = .005)
d R Squared = .488 (Adjusted R Squared = .458)
e R Squared = .811 (Adjusted R Squared = .800)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
needle_w_l	.353	.015	.323	.383
cone length (mm)	37.245	.806	35.637	38.852
Height/Age	.287	.007	.272	.301
DBH/Age	.396	.012	.373	.419
cone angle	28.009	2.012	23.995	32.022

2. species

Estimates

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
needle_w_l	1	.488	.021	.445	.530
	2	.168	.025	.118	.218
	3	.407	.025	.357	.457
	4	.482	.053	.376	.588
	5	.222	.034	.155	.289
cone length (mm)	1	38.326	1.138	36.056	40.597
	2	35.256	1.341	32.580	37.931
	3	41.757	1.341	39.082	44.433
	4	29.646	2.846	23.970	35.321
	5	41.238	1.800	37.648	44.827
Height/Age	1	.288	.010	.267	.308
	2	.292	.012	.268	.316
	3	.270	.012	.246	.295
	4	.315	.026	.264	.366
	5	.267	.016	.235	.300
DBH/Age	1	.486	.017	.453	.519
	2	.299	.020	.260	.338
	3	.332	.020	.293	.371
	4	.447	.041	.364	.530
	5	.416	.026	.363	.468
cone angle	1	-4.630	2.843	-10.300	1.039
	2	56.639	3.350	49.957	63.320
	3	40.247	3.350	33.565	46.928
	4	-12.260	7.107	-26.434	1.914
	5	60.050	4.495	51.085	69.014

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
needle_w_l	Contrast	1.355	4	.339	29.834	.000
	Error	.795	70	.011		
cone length (mm)	Contrast	767.783	4	191.946	5.926	.000
	Error	2267.370	70	32.391		
Height/Age	Contrast	.011	4	.003	1.095	.366
	Error	.184	70	.003		
DBH/Age	Contrast	.456	4	.114	16.653	.000
	Error	.480	70	.007		
cone angle	Contrast	60751.523	4	15187.881	75.180	.000
	Error	14141.426	70	202.020		

The F tests the effect of species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and upper	
needle_w_l	1	2	.319118(*)	.0329363	.000	.226892	.411345
		3	.080796	.0329363	.114	-.011431	.173023
		4	.005758	.0573781	1.000	-.154910	.166425
		5	.265417(*)	.0398668	.000	.153783	.377050
	2	1	-.319118(*)	.0329363	.000	-.411345	-.226892
		3	-.238322(*)	.0355161	.000	-.337773	-.138872
		4	-.313360(*)	.0588969	.000	-.478281	-.148440
		5	-.053702	.0420233	.705	-.171373	.063970
	3	1	-.080796	.0329363	.114	-.173023	.011431
		2	.238322(*)	.0355161	.000	.138872	.337773
		4	-.075038	.0588969	.708	-.239958	.089882
		5	.184621(*)	.0420233	.000	.066949	.302292
	4	1	-.005758	.0573781	1.000	-.166425	.154910
		2	.313360(*)	.0588969	.000	.148440	.478281
		3	.075038	.0588969	.708	-.089882	.239958
		5	.259659(*)	.0630349	.001	.083151	.436166
	5	1	-.265417(*)	.0398668	.000	-.377050	-.153783
		2	.053702	.0420233	.705	-.063970	.171373
		3	-.184621(*)	.0420233	.000	-.302292	-.066949
		4	-.259659(*)	.0630349	.001	-.436166	-.083151
cone length (mm)	1	2	3.071	1.7593	.413	-1.855	7.997
		3	-3.431	1.7593	.301	-8.357	1.495
		4	8.680(*)	3.0649	.046	.098	17.263

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and upper	
		5	-2.911	2.1295	.650	-8.874	3.052
	2	1	-3.071	1.7593	.413	-7.997	1.855
		3	-6.502(*)	1.8971	.009	-11.814	-1.190
		4	5.610	3.1460	.391	-3.200	14.419
		5	-5.982	2.2447	.070	-12.267	.303
	3	1	3.431	1.7593	.301	-1.495	8.357
		2	6.502(*)	1.8971	.009	1.190	11.814
		4	12.112(*)	3.1460	.002	3.302	20.921
		5	.520	2.2447	.999	-5.766	6.805
	4	1	-8.680(*)	3.0649	.046	-17.263	-.098
		2	-5.610	3.1460	.391	-14.419	3.200
		3	-12.112(*)	3.1460	.002	-20.921	-3.302
		5	-11.592(*)	3.3670	.008	-21.020	-2.163
	5	1	2.911	2.1295	.650	-3.052	8.874
		2	5.982	2.2447	.070	-.303	12.267
		3	-.520	2.2447	.999	-6.805	5.766
		4	11.592(*)	3.3670	.008	2.163	21.020
Height/Age	1	2	-.004646	.0158412	.998	-.049003	.039712
		3	.017189	.0158412	.814	-.027169	.061547
		4	-.027371	.0275967	.858	-.104646	.049904
		5	.020400	.0191744	.824	-.033291	.074092
	2	1	.004646	.0158412	.998	-.039712	.049003
		3	.021835	.0170820	.705	-.025998	.069667
		4	-.022725	.0283272	.929	-.102046	.056595
		5	.025046	.0202116	.729	-.031550	.081642
	3	1	-.017189	.0158412	.814	-.061547	.027169
		2	-.021835	.0170820	.705	-.069667	.025998
		4	-.044560	.0283272	.519	-.123881	.034761
		5	.003211	.0202116	1.000	-.053384	.059807
	4	1	.027371	.0275967	.858	-.049904	.104646
		2	.022725	.0283272	.929	-.056595	.102046
		3	.044560	.0283272	.519	-.034761	.123881
		5	.047771	.0303175	.518	-.037122	.132665
	5	1	-.020400	.0191744	.824	-.074092	.033291
		2	-.025046	.0202116	.729	-.081642	.031550
		3	-.003211	.0202116	1.000	-.059807	.053384
		4	-.047771	.0303175	.518	-.132665	.037122
DBH/Age	1	2	.186507(*)	.0255890	.000	.114854	.258160
		3	.153439(*)	.0255890	.000	.081786	.225092
		4	.038672	.0445783	.908	-.086154	.163499
		5	.070016	.0309734	.170	-.016714	.156746
	2	1	-.186507(*)	.0255890	.000	-.258160	-.114854
		3	-.033068	.0275933	.752	-.110333	.044198
		4	-.147835(*)	.0457583	.016	-.275965	-.019704
		5	-.116491(*)	.0326488	.006	-.207913	-.025069
	3	1	-.153439(*)	.0255890	.000	-.225092	-.081786
		2	.033068	.0275933	.752	-.044198	.110333

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and upper	
		4	-.114767	.0457583	.100	-.242897	.013363
		5	-.083423	.0326488	.090	-.174845	.007998
	4	1	-.038672	.0445783	.908	-.163499	.086154
		2	.147835(*)	.0457583	.016	.019704	.275965
		3	.114767	.0457583	.100	-.013363	.242897
		5	.031344	.0489732	.968	-.105789	.168476
	5	1	-.070016	.0309734	.170	-.156746	.016714
		2	.116491(*)	.0326488	.006	.025069	.207913
		3	.083423	.0326488	.090	-.007998	.174845
		4	-.031344	.0489732	.968	-.168476	.105789
cone angle	1	2	-61.268777(*)	4.3936509	.000	-73.571671	-48.965882
		3	-44.876602(*)	4.3936509	.000	-57.179497	-32.573707
		4	7.630215	7.6541431	.856	-13.802556	29.062987
		5	-64.679608(*)	5.3181624	.000	-79.571276	-49.787940
	2	1	61.268777(*)	4.3936509	.000	48.965882	73.571671
		3	16.392175(*)	4.7377956	.008	3.125622	29.658727
		4	68.898992(*)	7.8567452	.000	46.898904	90.899080
		5	-3.410831	5.6058354	.973	-19.108028	12.286365
	3	1	44.876602(*)	4.3936509	.000	32.573707	57.179497
		2	-16.392175(*)	4.7377956	.008	-29.658727	-3.125622
		4	52.506817(*)	7.8567452	.000	30.506729	74.506906
		5	-19.803006(*)	5.6058354	.006	-35.500203	-4.105809
	4	1	-7.630215	7.6541431	.856	-29.062987	13.802556
		2	-68.898992(*)	7.8567452	.000	-90.899080	-46.898904
		3	-52.506817(*)	7.8567452	.000	-74.506906	-30.506729
		5	-72.309823(*)	8.4087531	.000	-95.855618	-48.764028
	5	1	64.679608(*)	5.3181624	.000	49.787940	79.571276
		2	3.410831	5.6058354	.973	-12.286365	19.108028
		3	19.803006(*)	5.6058354	.006	4.105809	35.500203
		4	72.309823(*)	8.4087531	.000	48.764028	95.855618

Based on observed means.
* The mean difference is significant at the .05 level.

Cluster Analysis:

Quick Cluster

Cluster Membership

Case Number	species	Cluster	Distance
1	1	3	3.902
2	1	3	9.460
3	1	3	9.978
4	1	3	9.194
5	1	1	16.475
6	1	.	.
7	1	3	17.712
8	1	1	14.207
9	1	3	5.654
10	1	1	15.589
11	1	3	11.060
12	1	3	2.709
13	1	3	23.166
14	1	3	8.733
15	1	3	8.287
16	1	3	.588
17	1	3	4.715
18	1	3	1.475
19	1	3	14.610
20	1	3	13.152
21	1	3	5.066
22	1	3	1.074
23	1	3	1.682
24	1	3	6.561
25	1	1	14.514
26	1	3	12.456
27	1	3	19.432
28	1	3	8.263
29	1	3	19.719
30	1	3	14.675
31	2	.	.
32	2	.	.
33	2	2	4.075
34	2	2	7.408
35	2	2	16.442
36	2	.	.
37	2	.	.
38	2	2	5.367
39	2	2	7.799
40	2	2	10.869

Case Number	species	Cluster	Distance
41	2	2	6.741
42	2	2	8.664
43	2	2	3.188
44	2	.	.
45	2	.	.
46	2	.	.
47	2	2	10.222
48	2	2	5.512
49	2	2	11.806
50	2	2	9.171
51	2	.	.
52	2	.	.
53	2	2	7.385
54	2	2	9.012
55	2	.	.
56	2	.	.
57	2	.	.
58	2	2	6.974
59	2	2	12.177
60	2	2	13.612
61	3	1	11.946
62	3	1	6.781
63	3	3	15.977
64	3	1	19.513
65	3	1	17.032
66	3	2	22.107
67	3	1	9.796
68	3	1	7.105
69	3	1	1.195
70	3	.	.
71	3	1	14.150
72	3	2	2.531
73	3	.	.
74	3	2	10.435
75	3	1	4.820
76	3	1	18.258
77	3	2	4.799
78	3	2	1.737
79	3	2	14.350
80	3	1	1.339
81	3	1	7.551
82	3	2	4.987
83	3	2	18.561
84	3	2	11.089
85	3	2	18.403
86	3	2	1.448

Case Number	species	Cluster	Distance
87	3	2	23.331
88	3	2	10.673
89	3	2	14.583
90	3	1	15.021
91	.	.	.

ANOVA

	Cluster		Error			
	Mean Square	df	Mean Square	df	F	Sig.
needle_w_l	.473	2	.017	72	28.258	.000
cone length (mm)	77.637	2	39.998	72	1.941	.151
Height/Age	.004	2	.003	72	1.699	.190
DBH/Age	.169	2	.008	72	20.410	.000
cone angle	33756.255	2	102.506	72	329.310	.000

The F tests should be used only for descriptive purposes because the clusters have been chosen to maximize the differences among cases in different clusters. The observed significance levels are not corrected for this and thus cannot be interpreted as tests of the hypothesis that the cluster means are equal.

Wood Traits:

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	Species	Mean	Std. Deviation	N
MC % (OD-GR)	1	73.1685491	15.07510868	25
	2	60.1748146	12.58347879	28
	3	50.6340120	12.46159002	20
	4	76.1274276	18.77928001	4
	5	64.4717139	13.02086210	10
	Total	62.9427083	15.93643589	87
Density (g/cm3)	1	.4167148	.03763275	25
	2	.4324167	.04538827	28
	3	.4124949	.01938334	20
	4	.3958919	.03233939	4
	5	.3866175	.02535922	10
	Total	.4163814	.03792872	87

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	MC % (OD-GR)	6577.516(a)	4	1644.379	8.834	.000
	Density (g/cm3)	.018(b)	4	.005	3.500	.011
Intercept	MC % (OD-GR)	221456.279	1	221456.279	1189.697	.000
	Density (g/cm3)	8.784	1	8.784	6815.716	.000
Species	MC % (OD-GR)	6577.516	4	1644.379	8.834	.000
	Density (g/cm3)	.018	4	.005	3.500	.011
Error	MC % (OD-GR)	15263.903	82	186.145		
	Density (g/cm3)	.106	82	.001		
Total	MC % (OD-GR)	366516.673	87			
	Density (g/cm3)	15.207	87			
Corrected Total	MC % (OD-GR)	21841.419	86			
	Density (g/cm3)	.124	86			

a R Squared = .301 (Adjusted R Squared = .267)
b R Squared = .146 (Adjusted R Squared = .104)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MC % (OD-GR)	64.915	1.882	61.171	68.659
Density (g/cm3)	.409	.005	.399	.419

2. Species

Estimates

Dependent Variable	Species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
MC % (OD-GR)	1	73.169	2.729	67.740	78.597
	2	60.175	2.578	55.046	65.304
	3	50.634	3.051	44.565	56.703
	4	76.127	6.822	62.557	89.698
	5	64.472	4.314	55.889	73.055
Density (g/cm3)	1	.417	.007	.402	.431
	2	.432	.007	.419	.446
	3	.412	.008	.397	.428
	4	.396	.018	.360	.432
	5	.387	.011	.364	.409

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
MC % (OD-GR)	Contrast	6577.516	4	1644.379	8.834	.000
	Error	15263.903	82	186.145		
Density (g/cm3)	Contrast	.018	4	.005	3.500	.011
	Error	.106	82	.001		

The F tests the effect of Species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

Species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower	Upper
MC % (OD-GR)	1	2	12.9937345(*)	3.75417737	.007	2.5218125	23.4656565
		3	22.5345371(*)	4.09305073	.000	11.1173600	33.9517142
		4	-2.9588786	7.34725091	.994	-23.4533387	17.5355816
		5	8.6968352	5.10493116	.437	-5.5428865	22.9365569
	2	1	-12.9937345(*)	3.75417737	.007	-23.4656565	-2.5218125
		3	9.5408026	3.99440850	.129	-1.6012214	20.6828266
		4	-15.9526130	7.29275880	.195	-36.2950726	4.3898465
		5	-4.2968993	5.02618714	.912	-18.3169720	9.7231734
	3	1	-22.5345371(*)	4.09305073	.000	-33.9517142	-11.1173600
		2	-9.5408026	3.99440850	.129	-20.6828266	1.6012214
		4	-25.4934156(*)	7.47285404	.009	-46.3382338	-4.6485975
		5	-13.8377019	5.28410577	.076	-28.5772142	.9018104
	4	1	2.9588786	7.34725091	.994	-17.5355816	23.4533387
		2	15.9526130	7.29275880	.195	-4.3898465	36.2950726
		3	25.4934156(*)	7.47285404	.009	4.6485975	46.3382338
		5	11.6557138	8.07160489	.601	-10.8592631	34.1706907
	5	1	-8.6968352	5.10493116	.437	-22.9365569	5.5428865
		2	4.2968993	5.02618714	.912	-9.7231734	18.3169720
		3	13.8377019	5.28410577	.076	-.9018104	28.5772142
		4	-11.6557138	8.07160489	.601	-34.1706907	10.8592631
Density (g/cm3)	1	2	-.0157019	.00987802	.508	-.0432557	.0118519
		3	.0042199	.01076966	.995	-.0258210	.0342609
		4	.0208229	.01933214	.818	-.0331023	.0747481
		5	.0300973	.01343213	.175	-.0073704	.0675649
	2	1	.0157019	.00987802	.508	-.0118519	.0432557
		3	.0199219	.01051012	.328	-.0093951	.0492388
		4	.0365248	.01918876	.324	-.0170004	.0900500

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		5	.0457992(*)	.01322494	.007	.0089095	.0826889
	3	1	-.0042199	.01076966	.995	-.0342609	.0258210
		2	-.0199219	.01051012	.328	-.0492388	.0093951
		4	.0166030	.01966263	.916	-.0382441	.0714500
		5	.0258773	.01390358	.346	-.0129054	.0646600
	4	1	-.0208229	.01933214	.818	-.0747481	.0331023
		2	-.0365248	.01918876	.324	-.0900500	.0170004
		3	-.0166030	.01966263	.916	-.0714500	.0382441
		5	.0092744	.02123806	.992	-.0499672	.0685159
	5	1	-.0300973	.01343213	.175	-.0675649	.0073704
		2	-.0457992(*)	.01322494	.007	-.0826889	-.0089095
		3	-.0258773	.01390358	.346	-.0646600	.0129054
		4	-.0092744	.02123806	.992	-.0685159	.0499672

Based on observed means.
* The mean difference is significant at the .05 level.

Cluster Analysis:

Quick Cluster

Cluster Membership

Case Number	Species	Cluster	Distance
1	1	3	2.372
2	1	2	7.045
3	1	2	8.018
4	1	1	3.691
5	1	2	6.011
6	1	.	.
7	1	1	6.102
8	1	1	7.719
9	1	1	6.307
10	1	1	2.093
11	1	1	3.906
12	1	3	.600
13	1	1	.812
14	1	1	3.293
15	1	1	3.281
16	1	1	5.834
17	1	1	1.887
18	1	3	6.115
19	1	1	7.367
20	1	1	.859
21	1	3	8.423
22	1	1	10.164

23	1	1	6.463
24	1	3	3.668
25	1	3	9.595
26	1	3	27.052
27	1	2	1.230
28	1	3	4.119
29	1	3	.307
30	1	1	6.086
31	2	2	6.090
32	2	2	4.437
33	2	1	4.103
34	2	2	15.581
35	2	1	6.633
36	2	2	4.563
37	2	2	3.135
38	2	2	5.801
39	2	1	5.740
40	2	1	5.126
41	2	1	2.062
42	2	2	4.801
43	2	1	4.203
44	2	3	9.114
45	2	2	4.056
46	2	1	7.072
47	2	2	1.690
48	2	1	1.126
49	2	1	2.039
50	2	2	2.184
51	2	.	.
52	2	.	.
53	2	2	2.368
54	2	2	.855
55	2	2	5.679
56	2	2	4.829
57	2	3	7.800
58	2	1	.071
59	2	1	4.592
60	2	1	1.619
61	3	2	.779
62	3	1	4.209
63	3	1	7.269
64	3	2	3.059
65	3	2	13.958
66	3	2	.824
67	3	2	14.111
68	3	2	6.728
69	3	2	16.915

70	3	2	3.513
71	3	2	4.307
72	3	2	5.196
73	3	1	8.274
74	3	2	4.391
75	3	2	5.644
76	3	1	1.600
77	3	1	1.103
78	3	2	7.487
79	3	2	.617
80	3	2	2.829
81	3	1	1.833
82	3	2	2.543
83	3	2	1.451
84	3	1	9.588
85	3	3	3.384
86	3	2	8.650
87	3	1	8.424
88	3	1	8.055
89	3	2	4.933
90	3	1	2.651

ANOVA

	Cluster		Error			
	Mean Square	df	Mean Square	df	F	Sig.
MC % (OD-GR)	9003.456	2	45.649	84	197.233	.000
Density (g/cm3)	.005	2	.001	84	4.096	.020

The F tests should be used only for descriptive purposes because the clusters have been chosen to maximize the differences among cases in different clusters. The observed significance levels are not corrected for this and thus cannot be interpreted as tests of the hypothesis that the cluster means are equal.

Descriptive Statistics

	Species	Mean	Std. Deviation	N
MOE(ave)	1	7.9652000	1.50370234	5
	2	16.6204600	2.47957977	5
	3	12.1476800	3.25204041	3
	5	12.4672500	.50777338	2
	Total	12.2870560	4.16151138	15
ew/lw ratio	1	.7239786	.33833379	5
	2	.5721113	.23184168	5
	3	1.1803463	.33015285	3
	5	1.1078037	.84581096	2
	Total	.8158064	.42380675	15
Wall(ave)	1	2.4304920	.13864407	5
	2	2.7029860	.13377258	5
	3	2.3231033	.06165160	3
	5	2.3498650	.19176029	2
	Total	2.4890953	.20003411	15
mw dens	1	487.6642821	21.66650158	5
	2	542.9726672	31.51885557	5
	3	481.0143449	23.75394185	3
	5	470.1125129	60.95795948	2
	Total	502.4301871	40.94358205	15

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	MOE(ave)	187.407(a)	3	62.469	12.483	.001
	ew/lw ratio	.908(b)	3	.303	2.073	.162
	Wall(ave)	.367(c)	3	.122	6.985	.007
	mw dens	12773.402(d)	3	4257.801	4.379	.029
Intercept	MOE(ave)	1962.728	1	1962.728	392.210	.000
	ew/lw ratio	10.416	1	10.416	71.332	.000
	Wall(ave)	77.973	1	77.973	4447.661	.000
	mw dens	3184368.476	1	3184368.476	3274.913	.000
Species	MOE(ave)	187.407	3	62.469	12.483	.001
	ew/lw ratio	.908	3	.303	2.073	.162
	Wall(ave)	.367	3	.122	6.985	.007
	mw dens	12773.402	3	4257.801	4.379	.029
Error	MOE(ave)	55.047	11	5.004		
	ew/lw ratio	1.606	11	.146		

	Wall(ave)	.193	11	.018		
	mw dens	10695.875	11	972.352		
Total	MOE(ave)	2507.031	15			
	ew/lw ratio	12.498	15			
	Wall(ave)	93.494	15			
	mw dens	3810010.670	15			
Corrected Total	MOE(ave)	242.454	14			
	ew/lw ratio	2.515	14			
	Wall(ave)	.560	14			
	mw dens	23469.277	14			

a R Squared = .773 (Adjusted R Squared = .711)
b R Squared = .361 (Adjusted R Squared = .187)
c R Squared = .656 (Adjusted R Squared = .562)
d R Squared = .544 (Adjusted R Squared = .420)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MOE(ave)	12.300	.621	10.933	13.667
ew/lw ratio	.896	.106	.663	1.130
Wall(ave)	2.452	.037	2.371	2.533
mw dens	495.441	8.657	476.386	514.496

2. Species

Estimates

Dependent Variable	Species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
MOE(ave)	1	7.965	1.000	5.763	10.167
	2	16.620	1.000	14.419	18.822
	3	12.148	1.292	9.305	14.990
	5	12.467	1.582	8.986	15.949
ew/lw ratio	1	.724	.171	.348	1.100
	2	.572	.171	.196	.948
	3	1.180	.221	.695	1.666
	5	1.108	.270	.513	1.703
Wall(ave)	1	2.430	.059	2.300	2.561
	2	2.703	.059	2.573	2.833
	3	2.323	.076	2.155	2.491
	5	2.350	.094	2.144	2.556
mw dens	1	487.664	13.945	456.971	518.358
	2	542.973	13.945	512.279	573.666
	3	481.014	18.003	441.389	520.639
	5	470.113	22.049	421.582	518.643

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
MOE(ave)	Contrast	187.407	3	62.469	12.483	.001
	Error	55.047	11	5.004		
ew/lw ratio	Contrast	.908	3	.303	2.073	.162
	Error	1.606	11	.146		
Wall(ave)	Contrast	.367	3	.122	6.985	.007
	Error	.193	11	.018		
mw dens	Contrast	12773.402	3	4257.801	4.379	.029
	Error	10695.875	11	972.352		

The F tests the effect of Species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

Species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
MOE(ave)	1	2	-8.6552600(*)	1.41481915	.000	-12.9132259	-4.3972941
		3	-4.1824800	1.63369243	.104	-9.0991555	.7341955
		5	-4.5020500	1.87162981	.133	-10.1348094	1.1307094
	2	1	8.6552600(*)	1.41481915	.000	4.3972941	12.9132259
		3	4.4727800	1.63369243	.078	-.4438955	9.3894555
		5	4.1532100	1.87162981	.178	-1.4795494	9.7859694
	3	1	4.1824800	1.63369243	.104	-.7341955	9.0991555
		2	-4.4727800	1.63369243	.078	-9.3894555	.4438955
		5	-.3195700	2.04211554	.999	-6.4654144	5.8262744
	5	1	4.5020500	1.87162981	.133	-1.1307094	10.1348094
		2	-4.1532100	1.87162981	.178	-9.7859694	1.4795494
		3	.3195700	2.04211554	.999	-5.8262744	6.4654144
ew/lw ratio	1	2	.1518672	.24168193	.921	-.5754861	.8792205
		3	-.4563677	.27907026	.400	-1.2962430	.3835076
		5	-.3838251	.31971514	.639	-1.3460231	.5783729
	2	1	-.1518672	.24168193	.921	-.8792205	.5754861
		3	-.6082349	.27907026	.189	-1.4481102	.2316404
		5	-.5356924	.31971514	.380	-1.4978904	.4265057
	3	1	.4563677	.27907026	.400	-.3835076	1.2962430
		2	.6082349	.27907026	.189	-.2316404	1.4481102
		5	.0725426	.34883782	.997	-.9773015	1.1223867
	5	1	.3838251	.31971514	.639	-.5783729	1.3460231
		2	.5356924	.31971514	.380	-.4265057	1.4978904
		3	-.0725426	.34883782	.997	-1.1223867	.9773015
Wall(ave)	1	2	-.2724940(*)	.08374050	.033	-.5245150	-.0204730

		3	.1073887	.09669520	.691	-.1836202	.3983975
		5	.0806270	.11077827	.884	-.2527655	.4140195
	2	1	.2724940(*)	.08374050	.033	.0204730	.5245150
		3	.3798827(*)	.09669520	.011	.0888738	.6708915
		5	.3531210(*)	.11077827	.037	.0197285	.6865135
	3	1	-.1073887	.09669520	.691	-.3983975	.1836202
		2	-.3798827(*)	.09669520	.011	-.6708915	-.0888738
		5	-.0267617	.12086900	.996	-.3905227	.3369994
	5	1	-.0806270	.11077827	.884	-.4140195	.2527655
		2	-.3531210(*)	.11077827	.037	-.6865135	-.0197285
		3	.0267617	.12086900	.996	-.3369994	.3905227
mw dens	1	2	-55.3083851	19.72158441	.070	-114.6614366	4.0446664
		3	6.6499371	22.77252414	.991	-61.8850634	75.1849377
		5	17.5517692	26.08920390	.905	-60.9649378	96.0684761
	2	1	55.3083851	19.72158441	.070	-4.0446664	114.6614366
		3	61.9583222	22.77252414	.080	-6.5766783	130.4933228
		5	72.8601543	26.08920390	.072	-5.6565527	151.3768612
	3	1	-6.6499371	22.77252414	.991	-75.1849377	61.8850634
		2	-61.9583222	22.77252414	.080	-130.4933228	6.5766783
		5	10.9018320	28.46565517	.980	-74.7669187	96.5705827
	5	1	-17.5517692	26.08920390	.905	-96.0684761	60.9649378
		2	-72.8601543	26.08920390	.072	-151.3768612	5.6565527
		3	-10.9018320	28.46565517	.980	-96.5705827	74.7669187

Based on observed means.
* The mean difference is significant at the .05 level.

Quick Cluster

Cluster Membership

Case Number	Species	Cluster	Distance
1	1	.	.
2	1	.	.
3	1	.	.
4	1	.	.
5	1	1	13.893
6	1	.	.
7	1	.	.
8	1	1	19.445
9	1	.	.
10	1	.	.
11	1	.	.
12	1	.	.
13	1	3	5.575
14	1	.	.
15	1	1	12.632
16	1	.	.
17	1	.	.

18	1	.	.
19	1	.	.
20	1	.	.
21	1	.	.
22	1	.	.
23	1	.	.
24	1	.	.
25	1	.	.
26	1	.	.
27	1	.	.
28	1	.	.
29	1	.	.
30	1	1	9.776
31	2	.	.
32	2	.	.
33	2	2	7.809
34	2	.	.
35	2	2	16.396
36	2	.	.
37	2	.	.
38	2	.	.
39	2	1	8.972
40	2	.	.
41	2	.	.
42	2	.	.
43	2	.	.
44	2	.	.
45	2	.	.
46	2	.	.
47	2	.	.
48	2	2	8.875
49	2	.	.
50	2	.	.
51	2	.	.
52	2	.	.
53	2	.	.
54	2	.	.
55	2	.	.
56	2	.	.
57	2	.	.
58	2	.	.
59	2	.	.
60	2	1	8.965
61	3	.	.
62	3	.	.
63	3	.	.
64	3	.	.

65	3	.	.
66	3	3	11.216
67	3	3	15.386
68	3	.	.
69	3	.	.
70	3	.	.
71	3	.	.
72	3	.	.
73	3	.	.
74	3	.	.
75	3	.	.
76	3	.	.
77	3	.	.
78	3	.	.
79	3	.	.
80	3	1	5.864
81	3	.	.
82	3	.	.
83	3	.	.
84	3	.	.
85	3	.	.
86	3	.	.
87	3	.	.
88	3	3	27.282
89	3	.	.
90	3	1	10.285

ANOVA

	Cluster		Error			
	Mean Square	df	Mean Square	df	F	Sig.
ew/lw ratio	.434	2	.137	12	3.168	.079
mw dens	10483.096	2	208.590	12	50.257	.000
MOE(ave)	36.788	2	14.073	12	2.614	.114
Wall(ave)	.182	2	.016	12	11.059	.002

The F tests should be used only for descriptive purposes because the clusters have been chosen to maximize the differences among cases in different clusters. The observed significance levels are not corrected for this and thus cannot be interpreted as tests of the hypothesis that the cluster means are equal.

Fibre Traits:

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	species	Mean	Std. Deviation	N
Fibre length (20-40)	1	2.4710	.21279	5
	2	2.5330	.16312	5
	3	2.4350	.02500	3
	5	2.3100	.21213	2
	Total	2.4630	.17067	15
coarseness (20-40)	1	.20020	.027813	5
	2	.20930	.015446	5
	3	.19767	.008780	3
	5	.22375	.003889	2
	Total	.20587	.019418	15

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Fibre length (20-40)	.074(a)	3	.025	.813	.513
	coarseness (20-40)	.001(b)	3	.000	.922	.462
Intercept	Fibre length (20-40)	77.062	1	77.062	2539.487	.000
	coarseness (20-40)	.560	1	.560	1459.929	.000
species	Fibre length (20-40)	.074	3	.025	.813	.513
	coarseness (20-40)	.001	3	.000	.922	.462
Error	Fibre length (20-40)	.334	11	.030		
	coarseness (20-40)	.004	11	.000		
Total	Fibre length (20-40)	91.403	15			
	coarseness (20-40)	.641	15			
Corrected Total	Fibre length (20-40)	.408	14			
	coarseness (20-40)	.005	14			

a R Squared = .181 (Adjusted R Squared = -.042)
b R Squared = .201 (Adjusted R Squared = -.017)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Fibre length (20-40)	2.437	.048	2.331	2.544
coarseness (20-40)	.208	.005	.196	.220

2. species

Estimates

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Fibre length (20-40)	1	2.471	.078	2.300	2.642
	2	2.533	.078	2.362	2.704
	3	2.435	.101	2.214	2.656
	5	2.310	.123	2.039	2.581
coarseness (20-40)	1	.200	.009	.181	.219
	2	.209	.009	.190	.229
	3	.198	.011	.173	.223
	5	.224	.014	.193	.254

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Fibre length (20-40)	Contrast	.074	3	.025	.813	.513
	Error	.334	11	.030		
coarseness (20-40)	Contrast	.001	3	.000	.922	.462
	Error	.004	11	.000		

The F tests the effect of species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Fibre length (20-40)	1	2	-.0620	.11017	.941	-.3936	.2696
		3	.0360	.12722	.992	-.3469	.4189
		5	.1610	.14575	.694	-.2776	.5996
	2	1	.0620	.11017	.941	-.2696	.3936
		3	.0980	.12722	.866	-.2849	.4809
		5	.2230	.14575	.454	-.2156	.6616
	3	1	-.0360	.12722	.992	-.4189	.3469
		2	-.0980	.12722	.866	-.4809	.2849

		5	.1250	.15902	.859	-.3536	.6036
	5	1	-.1610	.14575	.694	-.5996	.2776
		2	-.2230	.14575	.454	-.6616	.2156
		3	-.1250	.15902	.859	-.6036	.3536
coarseness (20-40)	1	2	-.00910	.012385	.881	-.04637	.02817
		3	.00253	.014300	.998	-.04050	.04557
		5	-.02355	.016383	.504	-.07286	.02576
	2	1	.00910	.012385	.881	-.02817	.04637
		3	.01163	.014300	.847	-.03140	.05467
		5	-.01445	.016383	.814	-.06376	.03486
	3	1	-.00253	.014300	.998	-.04557	.04050
		2	-.01163	.014300	.847	-.05467	.03140
		5	-.02608	.017876	.492	-.07988	.02771
	5	1	.02355	.016383	.504	-.02576	.07286
		2	.01445	.016383	.814	-.03486	.06376
		3	.02608	.017876	.492	-.02771	.07988

Based on observed means.

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	species	Mean	Std. Deviation	N
Fibre length (40-60)	1	2.6240	.25967	10
	2	2.6380	.17968	26
	3	2.5246	.16489	13
	4	2.2850	.	1
	Total	2.5986	.19982	50
Fibre length (60-80)	1	2.6515	.27564	10
	2	2.6475	.17825	26
	3	2.5378	.16869	13
	4	2.6050	.	1
	Total	2.6189	.19884	50
Fibre coarseness (40-60)	1	.19405	.017940	10
	2	.21152	.021008	26
	3	.19314	.017968	13
	4	.15800	.	1
	Total	.20217	.022014	50
Fibre coarseness (60-80)	1	.2025	.02486	10
	2	.2139	.01923	26
	3	.1894	.01854	13
	4	.1850	.	1
	Total	.2047	.02240	50

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Fibre length (40-60)	.216(a)	3	.072	1.905	.142
	Fibre length (60-80)	.118(b)	3	.039	.991	.405
	Fibre coarseness (40-60)	.006(c)	3	.002	5.117	.004
	Fibre coarseness (60-80)	.006(d)	3	.002	4.590	.007
Intercept	Fibre length (40-60)	83.461	1	83.461	2206.177	.000
	Fibre length (60-80)	89.709	1	89.709	2267.886	.000
	Fibre coarseness (40-60)	.471	1	.471	1217.269	.000
	Fibre coarseness (60-80)	.514	1	.514	1250.289	.000
species	Fibre length (40-60)	.216	3	.072	1.905	.142
	Fibre length (60-80)	.118	3	.039	.991	.405
	Fibre coarseness (40-60)	.006	3	.002	5.117	.004
	Fibre coarseness (60-80)	.006	3	.002	4.590	.007
Error	Fibre length (40-60)	1.740	46	.038		
	Fibre length (60-80)	1.820	46	.040		
	Fibre coarseness (40-60)	.018	46	.000		
	Fibre coarseness (60-80)	.019	46	.000		
Total	Fibre length (40-60)	339.603	50			
	Fibre length (60-80)	344.874	50			
	Fibre coarseness (40-60)	2.067	50			
	Fibre coarseness (60-80)	2.119	50			
Corrected Total	Fibre length (40-60)	1.956	49			
	Fibre length (60-80)	1.937	49			
	Fibre coarseness (40-60)	.024	49			
	Fibre coarseness (60-80)	.025	49			

a R Squared = .111 (Adjusted R Squared = .053)
b R Squared = .061 (Adjusted R Squared = -.001)
c R Squared = .250 (Adjusted R Squared = .201)
d R Squared = .230 (Adjusted R Squared = .180)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Fibre length (40-60)	2.518	.054	2.410	2.626
Fibre length (60-80)	2.610	.055	2.500	2.721
Fibre coarseness (40-60)	.189	.005	.178	.200
Fibre coarseness (60-80)	.198	.006	.186	.209

2. species

Estimates

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound

Fibre length (40-60)	1	2.624	.062	2.500	2.748
	2	2.638	.038	2.561	2.715
	3	2.525	.054	2.416	2.633
	4	2.285	.195	1.893	2.677
Fibre length (60-80)	1	2.652	.063	2.525	2.778
	2	2.648	.039	2.569	2.726
	3	2.538	.055	2.427	2.649
	4	2.605	.199	2.205	3.005
Fibre coarseness (40-60)	1	.194	.006	.182	.207
	2	.212	.004	.204	.219
	3	.193	.005	.182	.204
	4	.158	.020	.118	.198
Fibre coarseness (60-80)	1	.203	.006	.190	.215
	2	.214	.004	.206	.222
	3	.189	.006	.178	.201
	4	.185	.020	.144	.226

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Fibre length (40-60)	Contrast	.216	3	.072	1.905	.142
	Error	1.740	46	.038		
Fibre length (60-80)	Contrast	.118	3	.039	.991	.405
	Error	1.820	46	.040		
Fibre coarseness (40-60)	Contrast	.006	3	.002	5.117	.004
	Error	.018	46	.000		
Fibre coarseness (60-80)	Contrast	.006	3	.002	4.590	.007
	Error	.019	46	.000		

The F tests the effect of species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Cluster Analysis:

Quick Cluster

Cluster Membership

Case Number	species	Cluster	Distance
1	1	.	.
2	1	3	.153
3	1	2	.241
4	1	.	.
5	1	.	.
6	1	.	.
7	1	3	.302

Case Number	species	Cluster	Distance
8	1	2	.158
9	1	1	.149
10	1	3	.073
11	1	2	.254
12	1	.	.
13	1	.	.
14	1	.	.
15	1	.	.
16	1	1	.131
17	1	3	.246
18	1	.	.
19	1	1	.234
20	1	.	.
21	1	.	.
22	1	.	.
23	1	.	.
24	1	.	.
25	1	.	.
26	1	.	.
27	1	.	.
28	1	.	.
29	1	.	.
30	1	1	.106
31	2	3	.250
32	2	3	.094
33	2	1	.093
34	2	2	.272
35	2	.	.
36	2	1	.149
37	2	3	.132
38	2	3	.080
39	2	3	.130
40	2	3	.290
41	2	1	.117
42	2	1	.114
43	2	3	.152
44	2	3	.175
45	2	1	.184
46	2	3	.133
47	2	1	.082
48	2	1	.280
49	2	1	.091
50	2	2	.136
51	2	.	.
52	2	.	.
53	2	3	.032

Case Number	species	Cluster	Distance
54	2	1	.078
55	2	2	.069
56	2	3	.155
57	2	.	.
58	2	1	.068
59	2	1	.140
60	2	1	.106
61	3	1	.049
62	3	.	.
63	3	1	.068
64	3	1	.120
65	3	3	.043
66	3	3	.161
67	3	1	.050
68	3	3	.114
69	3	2	.063
70	3	.	.
71	3	1	.154
72	3	.	.
73	3	.	.
74	3	.	.
75	3	1	.131
76	3	.	.
77	3	.	.
78	3	1	.134
79	3	1	.149
80	3	2	.178
81	3	.	.
82	3	.	.
83	3	.	.
84	3	.	.
85	3	.	.
86	3	.	.
87	3	.	.
88	3	.	.
89	3	.	.
90	3	.	.
91	.	.	.
92	.	.	.
93	.	.	.

ANOVA

	Cluster		Error		F	Sig.
	Mean Square	df	Mean Square	df		
Fibre length (40-60)	.733	2	.010	47	70.339	.000
Fibre length (60-80)	.617	2	.015	47	41.328	.000
Fibre coarseness (40-60)	.002	2	.000	47	5.619	.006
Fibre coarseness (60-80)	.003	2	.000	47	7.349	.002

The F tests should be used only for descriptive purposes because the clusters have been chosen to maximize the differences among cases in different clusters. The observed significance levels are not corrected for this and thus cannot be interpreted as tests of the hypothesis that the cluster means are equal.

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	Species	Mean	Std. Deviation	N
Crs(ave)	1	358.77780	21.400743	5
	2	395.49000	20.465508	5
	3	343.60200	7.950180	3
	5	368.22050	19.627163	2
	Total	369.23907	26.706810	15
MFA(ave)	1	27.590220	4.7595925	5
	2	13.733358	3.7984525	5
	3	17.848300	6.8601941	3
	5	17.015000	.4792770	2
	Total	19.612853	7.3511658	15
mw mfa	1	21.876273	5.7484767	5
	2	12.970088	4.5052271	5
	3	14.391544	6.8823237	3
	5	13.581224	4.8868964	2
	Total	16.304592	6.3729798	15

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Crs(ave)	5966.601(a)	3	1988.867	5.444	.015
	MFA(ave)	513.873(b)	3	171.291	7.764	.005
	mw mfa	236.625(c)	3	78.875	2.613	.104
Intercept	Crs(ave)	1742773.595	1	1742773.595	4770.028	.000
	MFA(ave)	4706.303	1	4706.303	213.322	.000
	mw mfa	3199.656	1	3199.656	106.018	.000
species	Crs(ave)	5966.601	3	1988.867	5.444	.015
	MFA(ave)	513.873	3	171.291	7.764	.005
	mw mfa	236.625	3	78.875	2.613	.104
Error	Crs(ave)	4018.952	11	365.359		
	MFA(ave)	242.682	11	22.062		
	mw mfa	331.983	11	30.180		
Total	Crs(ave)	2055047.877	15			
	MFA(ave)	6526.515	15			
	mw mfa	4556.204	15			
Corrected Total	Crs(ave)	9985.552	14			
	MFA(ave)	756.555	14			
	mw mfa	568.608	14			

a R Squared = .598 (Adjusted R Squared = .488)
b R Squared = .679 (Adjusted R Squared = .592)
c R Squared = .416 (Adjusted R Squared = .257)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Crs(ave)	366.523	5.307	354.842	378.203
MFA(ave)	19.047	1.304	16.176	21.917
mw mfa	15.705	1.525	12.348	19.062

2. species

Estimates

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Crs(ave)	1	358.778	8.548	339.963	377.592
	2	395.490	8.548	376.676	414.304
	3	343.602	11.036	319.313	367.891
	5	368.220	13.516	338.472	397.969
MFA(ave)	1	27.590	2.101	22.967	32.214
	2	13.733	2.101	9.110	18.357
	3	17.848	2.712	11.880	23.817
	5	17.015	3.321	9.705	24.325
mw mfa	1	21.876	2.457	16.469	27.284
	2	12.970	2.457	7.563	18.378
	3	14.392	3.172	7.411	21.373
	5	13.581	3.885	5.031	22.131

Multivariate Tests

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Crs(ave)	Contrast	5966.601	3	1988.867	5.444	.015
	Error	4018.952	11	365.359		
MFA(ave)	Contrast	513.873	3	171.291	7.764	.005
	Error	242.682	11	22.062		
mw mfa	Contrast	236.625	3	78.875	2.613	.104
	Error	331.983	11	30.180		

The F tests the effect of species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and upper	
Crs(ave)	1	2	-36.71220(*)	12.088991	.048	-73.09460	-.32980
		3	15.17580	13.959164	.704	-26.83497	57.18657
		5	-9.44270	15.992231	.933	-57.57208	38.68668
	2	1	36.71220(*)	12.088991	.048	.32980	73.09460
		3	51.88800(*)	13.959164	.015	9.87723	93.89877
		5	27.26950	15.992231	.366	-20.85988	75.39888
	3	1	-15.17580	13.959164	.704	-57.18657	26.83497
		2	-51.88800(*)	13.959164	.015	-93.89877	-9.87723
		5	-24.61850	17.448955	.518	-77.13196	27.89496
	5	1	9.44270	15.992231	.933	-38.68668	57.57208
		2	-27.26950	15.992231	.366	-75.39888	20.85988
		3	24.61850	17.448955	.518	-27.89496	77.13196
MFA(ave)	1	2	13.856862(*)	2.9706570	.003	4.916528	22.797196
		3	9.741920	3.4302192	.066	-.581489	20.065329
		5	10.575220	3.9298098	.084	-1.251731	22.402171
	2	1	-13.856862(*)	2.9706570	.003	-22.797196	-4.916528
		3	-4.114942	3.4302192	.640	-14.438351	6.208467
		5	-3.281642	3.9298098	.837	-15.108593	8.545309
	3	1	-9.741920	3.4302192	.066	-20.065329	.581489
		2	4.114942	3.4302192	.640	-6.208467	14.438351
		5	.833300	4.2877740	.997	-12.070961	13.737561
	5	1	-10.575220	3.9298098	.084	-22.402171	1.251731
		2	3.281642	3.9298098	.837	-8.545309	15.108593
		3	-.833300	4.2877740	.997	-13.737561	12.070961
mw mfa	1	2	8.906185	3.4744927	.104	-1.550467	19.362837

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and upper	
		3	7.484729	4.011998 6	.296	-4.589572	19.559031
		5	8.295049	4.596321 8	.322	-5.537802	22.127899
	2	1	-8.906185	3.474492 7	.104	-19.362837	1.550467
		3	-1.421456	4.011998 6	.984	-13.495758	10.652846
		5	-.611136	4.596321 8	.999	-14.443987	13.221714
	3	1	-7.484729	4.011998 6	.296	-19.559031	4.589572
		2	1.421456	4.011998 6	.984	-10.652846	13.495758
		5	.810320	5.014998 3	.998	-14.282558	15.903197
	5	1	-8.295049	4.596321 8	.322	-22.127899	5.537802
		2	.611136	4.596321 8	.999	-13.221714	14.443987
		3	-.810320	5.014998 3	.998	-15.903197	14.282558

Based on observed means.
* The mean difference is significant at the .05 level.

Quick Cluster

Cluster Membership

Case Number	species	Cluster	Distance
1	1	.	.
2	1	.	.
3	1	.	.
4	1	.	.
5	1	1	9.409
6	1	.	.
7	1	.	.
8	1	2	11.064
9	1	.	.
10	1	.	.
11	1	.	.
12	1	.	.
13	1	2	24.106
14	1	.	.
15	1	2	8.771
16	1	.	.
17	1	.	.
18	1	.	.
19	1	.	.
20	1	.	.

Case Number	species	Cluster	Distance
21	1	.	.
22	1	.	.
23	1	.	.
24	1	.	.
25	1	.	.
26	1	.	.
27	1	.	.
28	1	.	.
29	1	.	.
30	1	2	10.252
31	2	.	.
32	2	.	.
33	2	3	5.517
34	2	.	.
35	2	3	11.155
36	2	.	.
37	2	.	.
38	2	.	.
39	2	3	10.440
40	2	.	.
41	2	.	.
42	2	.	.
43	2	.	.
44	2	.	.
45	2	.	.
46	2	.	.
47	2	.	.
48	2	2	16.589
49	2	.	.
50	2	.	.
51	2	.	.
52	2	.	.
53	2	.	.
54	2	.	.
55	2	.	.
56	2	.	.
57	2	.	.
58	2	.	.
59	2	.	.
60	2	2	11.717
61	3	.	.
62	3	.	.
63	3	.	.
64	3	.	.
65	3	.	.
66	3	1	10.099

Case Number	species	Cluster	Distance
67	3	2	18.951
68	3	.	.
69	3	.	.
70	3	.	.
71	3	.	.
72	3	.	.
73	3	.	.
74	3	.	.
75	3	.	.
76	3	.	.
77	3	.	.
78	3	.	.
79	3	.	.
80	3	1	8.609
81	3	.	.
82	3	.	.
83	3	.	.
84	3	.	.
85	3	.	.
86	3	.	.
87	3	.	.
88	3	2	16.217
89	3	.	.
90	3	2	15.096
91	.	.	.
92	.	.	.
93	.	.	.

ANOVA

	Cluster		Error		F	Sig.
	Mean Square	df	Mean Square	df		
Crs(ave)	4170.257	2	137.087	12	30.421	.000
MFA(ave)	106.087	2	45.365	12	2.339	.139
mw mfa	40.743	2	40.593	12	1.004	.395

The F tests should be used only for descriptive purposes because the clusters have been chosen to maximize the differences among cases in different clusters. The observed significance levels are not corrected for this and thus cannot be interpreted as tests of the hypothesis that the cluster means are equal.

Chemical Extractives Analysis:

General Linear Model

Between-Subjects Factors

Descriptive Statistics

	species	Mean	Std. Deviation	N
Region1	1	997978.38	428355.902	26
	2	1053199.27	447762.663	33
	3	860841.72	301837.362	29
	4	985693.75	761049.831	4
	5	1217974.50	506739.541	10
	Total	997940.63	430670.259	102
Region2	1	948036.31	349465.907	26
	2	881341.76	340557.426	33
	3	725037.72	224907.477	29
	4	735184.00	380531.451	4
	5	853390.90	263895.120	10
	Total	845430.99	315096.810	102
Region3	1	1612528.42	640714.262	26
	2	1448967.24	518643.429	33
	3	1165961.52	375878.762	29
	4	1051526.25	427732.132	4
	5	1320014.80	467214.055	10
	Total	1381968.58	533051.376	102
Region4	1	1722941.69	742149.088	26
	2	1485725.76	543850.910	33
	3	1168943.10	442592.632	29
	4	1017864.75	403435.330	4
	5	1177970.70	402503.849	10
	Total	1407607.35	599001.854	102
Region5	1	1920144.96	1280142.437	26
	2	1130924.24	386361.838	33
	3	1159925.69	484987.925	29
	4	985544.25	351493.939	4
	5	908956.20	319987.687	10
	Total	1312880.9	814736.008	102

	species	Mean	Std. Deviation	N
		1		
Region6	1	1509703.77	2309683.350	26
	2	708134.91	283325.246	33
	3	733267.24	473800.532	29
	4	620370.25	271473.828	4
	5	550269.90	195104.922	10
	Total	900683.14	1242825.080	102
Region7	1	9937021.04	7952795.360	26
	2	6061167.15	3498435.670	33
	3	6541355.48	4182090.094	29
	4	6246992.75	3921525.033	4
	5	4615793.70	2465676.856	10
	Total	7051238.04	5341506.677	102
Region8	1	3623652.92	3044049.284	26
	2	2018649.18	1008220.541	33
	3	1939260.10	1175138.581	29
	4	1743232.75	645157.098	4
	5	1449192.60	449563.067	10
	Total	2338566.66	1904099.259	102
Region9	1	1714198.62	1099564.966	26
	2	1441426.52	829546.352	33
	3	917772.48	454975.201	29
	4	810152.25	329005.744	4
	5	867292.70	464402.055	10
	Total	1281031.15	847018.825	102
Region10	1	1110107.00	825216.445	26
	2	687199.76	287924.796	33
	3	610353.41	271797.278	29
	4	559390.25	194684.175	4
	5	505814.90	158610.674	10
	Total	750356.21	516028.510	102
Region11	1	2284147.38	1752018.934	26
	2	1787259.79	1236690.029	33
	3	1132913.10	817914.143	29
	4	985906.75	521702.389	4

	species	Mean	Std. Deviation	N
	5	857762.00	518772.872	10
	Total	1605324.82	1313745.529	102
Region12	1	1449994.85	1294889.292	26
	2	775934.48	324133.042	33
	3	801053.34	394551.126	29
	4	784444.50	340890.314	4
	5	535200.90	201585.519	10
	Total	931627.82	772628.644	102
Region13	1	9658655.23	8787816.679	26
	2	3344723.76	1901392.627	33
	3	4373912.00	3645166.747	29
	4	4622287.25	1924071.209	4
	5	2266540.70	1512186.775	10
	Total	5191165.92	5617941.768	102
Region14	1	14100323.65	18213747.873	26
	2	3456719.18	2644550.713	33
	3	4501569.14	4784899.196	29
	4	3907738.75	2595726.510	4
	5	1884505.20	1613201.508	10
	Total	6330408.43	10606520.232	102
Region15	1	22351318.65	21493927.287	26
	2	7512855.97	5001175.906	33
	3	13591462.34	11702861.422	29
	4	10963865.75	6168584.729	4
	5	4568190.10	3774673.585	10
	Total	12870081.41	14222579.213	102
Region16	1	13284278.50	16795088.689	26
	2	2501626.97	1770229.440	33
	3	3911247.86	4111163.343	29
	4	3848104.25	2921946.240	4
	5	1399430.80	1158952.273	10
	Total	5595665.14	9843617.906	102

	species	Mean	Std. Deviation	N
Region17	1	9663135.08	7944763.196	26
	2	3516890.39	2121205.540	33
	3	4708377.52	4308624.161	29
	4	5310747.75	2255043.379	4
	5	2707901.30	1579604.357	10
	Total	5413371.05	5409370.261	102
Region18	1	5072989.27	4674742.309	26
	2	1886393.03	1057982.563	33
	3	2743960.62	2482266.431	29
	4	2759073.75	1158995.351	4
	5	1337661.70	720726.288	10
	Total	2922906.48	3059142.982	102
Region19	1	1639267.69	1641703.648	26
	2	679461.61	305089.755	33
	3	890697.38	659672.842	29
	4	872146.50	318124.448	4
	5	542201.40	234513.486	10
	Total	978274.68	994126.018	102
Region20	1	2561523.23	2207244.729	26
	2	1141063.39	613938.531	33
	3	1775778.93	1703171.701	29
	4	1448148.00	493069.229	4
	5	998828.00	548517.525	10
	Total	1681697.62	1584044.604	102

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Region1	11306022911 50.090(a)	4	28265057278 7.522	1.558	.192
	Region2	78587319057 8.695(b)	4	19646829764 4.674	2.062	.092
	Region3	33584986836 02.903(c)	4	83962467090 0.726	3.214	.016
	Region4	55734966004 08.180(d)	4	13933741501 02.045	4.407	.003
	Region5	13419194441 344.650(e)	4	33547986103 36.164	6.068	.000
	Region6	13222038461 101.650(f)	4	33055096152 75.414	2.246	.070
	Region7	31830982311 0400.500(g)	4	79577455777 600.100	3.011	.022
	Region8	60266549756 842.100(h)	4	15066637439 210.540	4.777	.001
	Region9	12152917318 508.290(i)	4	30382293296 27.073	4.887	.001
	Region10	48088639603 85.920(j)	4	12022159900 96.482	5.280	.001
	Region11	26668341652 087.620(k)	4	66670854130 21.900	4.380	.003
	Region12	99388832622 24.790(h)	4	24847208155 56.199	4.787	.001
	Region13	73762651870 2870.000(l)	4	18440662967 5717.500	7.301	.000
	Region14	21603109241 17532.000(m)	4	54007773102 9383.000	5.693	.000
	Region15	40031758262 69166.000(n)	4	10007939565 67291.000	5.909	.000
	Region16	21234758310 04049.000(o)	4	53086895775 1012.000	6.720	.000
	Region17	67591318680 2739.000(p)	4	16897829670 0684.700	7.191	.000
	Region18	18181414212 5710.100(q)	4	45453535531 427.500	5.776	.000
	Region19	16475326716 440.670(r)	4	41188316791 10.168	4.794	.001
	Region20	34909816209 001.690(s)	4	87274540522 50.420	3.874	.006
Intercept	Region1	57739468854 360.900	1	57739468854 360.900	318.177	.000
	Region2	37869769583 693.790	1	37869769583 693.790	397.464	.000
	Region3	96077296091 201.400	1	96077296091 201.400	367.778	.000
	Region4	95334687593 180.900	1	95334687593 180.900	301.558	.000
	Region5	82244441695 856.100	1	82244441695 856.100	148.771	.000
	Region6	37482384642 182.600	1	37482384642 182.600	25.464	.000
	Region7	24616044932 12677.000	1	24616044932 12677.000	93.148	.000
	Region8	25610478185 7150.000	1	25610478185 7150.000	81.205	.000
	Region9	72967203973	1	72967203973	117.360	.000

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
		585.200		585.200		
	Region10	26609740006 508.210	1	26609740006 508.210	116.868	.000
	Region11	10959612182 6841.600	1	10959612182 6841.600	72.000	.000
	Region12	41684030965 137.360	1	41684030965 137.360	80.299	.000
	Region13	12991682305 70478.000	1	12991682305 70478.000	51.435	.000
	Region14	17113618300 88741.000	1	17113618300 88741.000	18.040	.000
	Region15	76769297771 87310.000	1	76769297771 87310.000	45.331	.000
	Region16	13728431275 10619.000	1	13728431275 10619.000	17.378	.000
	Region17	14808147886 43031.000	1	14808147886 43031.000	63.014	.000
	Region18	42017272142 1110.400	1	42017272142 1110.400	53.390	.000
	Region19	47169150380 924.300	1	47169150380 924.300	54.899	.000
	Region20	13858005327 3649.800	1	13858005327 3649.800	61.515	.000
Species	Region1	11306022911 50.100	4	28265057278 7.525	1.558	.192
	Region2	78587319057 8.698	4	19646829764 4.675	2.062	.092
	Region3	33584986836 02.874	4	83962467090 0.719	3.214	.016
	Region4	55734966004 08.150	4	13933741501 02.039	4.407	.003
	Region5	13419194441 344.620	4	33547986103 36.157	6.068	.000
	Region6	13222038461 101.770	4	33055096152 75.444	2.246	.070
	Region7	31830982311 0402.600	4	79577455777 600.600	3.011	.022
	Region8	60266549756 842.400	4	15066637439 210.600	4.777	.001
	Region9	12152917318 508.300	4	30382293296 27.076	4.887	.001
	Region10	48088639603 85.920	4	12022159900 96.482	5.280	.001
	Region11	26668341652 087.710	4	66670854130 21.920	4.380	.003
	Region12	99388832622 24.740	4	24847208155 56.186	4.787	.001
	Region13	73762651870 2871.000	4	18440662967 5717.900	7.301	.000
	Region14	21603109241 17536.000	4	54007773102 9384.000	5.693	.000
	Region15	40031758262 69172.000	4	10007939565 67293.000	5.909	.000
	Region16	21234758310 04040.000	4	53086895775 1010.000	6.720	.000
	Region17	67591318680 2738.000	4	16897829670 0684.700	7.191	.000
	Region18	18181414212 5709.700	4	45453535531 427.400	5.776	.000

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
	Region19	16475326716440.670	4	4118831679110.169	4.794	.001
	Region20	34909816209001.740	4	8727454052250.430	3.874	.006
Error	Region1	17602561750761.740	97	1814697087700.740		
	Region2	92420127504 26.290	97	95278481963.158		
	Region3	25340022061515.980	97	2612373408400.371		
	Region4	30665628723103.140	97	3161405023000.032		
	Region5	53624076624689.500	97	5528255322103.294		
	Region6	14278399354 9632.300	97	1471999933501.365		
	Region7	25633912280 95911.000	97	2642671369100.430		
	Region8	30591844294 6264.700	97	3153798380889.327		
	Region9	60308612574924.400	97	6217382739608.294		
	Region10	22085963728118.730	97	2276903477102.564		
	Region11	14765031703 2455.100	97	1522168216829.434		
	Region12	50353573951046.000	97	5191090098004.598		
	Region13	24500617217 72090.000	97	2525836826500691.650		
	Region14	92020144900 46860.000	97	9486612876300369.700		
	Region15	16427281880682210.000	97	1693534214500332.100		
	Region16	76631023301 76800.000	97	7900105495000276.300		
	Region17	22794767616 53389.000	97	2349976042900416.380		
	Region18	76337979239 1340.000	97	7869894766900021.030		
	Region19	83341613854499.600	97	8591918954007.213		
	Region20	21851911172 1106.300	97	2252774347600040.272		
Total	Region1	12031348462 5472.000	102			
	Region2	82932748977764.900	102			
	Region3	22350191022 5825.000	102			
	Region4	23833768824 9026.000	102			
	Region5	24285621249 0599.000	102			
	Region6	23875150361 1738.000	102			
	Region7	79531367555 45900.000	102			
	Region8	92401218157	102			

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
		9307.000				
	Region9	23984769146 6387.000	102			
	Region10	84324340130 532.900	102			
	Region11	43717957316 6595.900	102			
	Region12	14882135817 3833.900	102			
	Region13	59364050102 51020.000	102			
	Region14	15449880646 779600.000	102			
	Region15	37325635252 587350.000	102			
	Region16	12980347930 666380.000	102			
	Region17	59444577321 21220.000	102			
	Region18	18166189284 15133.000	102			
	Region19	19743311751 8551.000	102			
	Region20	54189582940 4487.000	102			
Corrected Total	Region1	18733164041 911.830	101			
	Region2	10027885941 004.990	101			
	Region3	28698520745 118.890	101			
	Region4	36239125323 511.320	101			
	Region5	67043271066 034.200	101			
	Region6	15600603201 0734.000	101			
	Region7	28817010512 06312.000	101			
	Region8	36618499270 3106.900	101			
	Region9	72461529893 432.700	101			
	Region10	26894827688 504.660	101			
	Region11	17431865868 4542.700	101			
	Region12	60292457213 270.800	101			
	Region13	31876882404 74960.000	101			
	Region14	11362325414 164400.000	101			
	Region15	20430457706 951380.000	101			
	Region16	97865781611 80850.000	101			
	Region17	29553899484 56128.000	101			

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
	Region18	94519393451 7050.000	101			
	Region19	99816940570 940.300	101			
	Region20	25342892793 0108.000	101			

a R Squared = .060 (Adjusted R Squared = .022)
b R Squared = .078 (Adjusted R Squared = .040)
c R Squared = .117 (Adjusted R Squared = .081)
d R Squared = .154 (Adjusted R Squared = .119)
e R Squared = .200 (Adjusted R Squared = .167)
f R Squared = .085 (Adjusted R Squared = .047)
g R Squared = .110 (Adjusted R Squared = .074)
h R Squared = .165 (Adjusted R Squared = .130)
i R Squared = .168 (Adjusted R Squared = .133)
j R Squared = .179 (Adjusted R Squared = .145)
k R Squared = .153 (Adjusted R Squared = .118)
l R Squared = .231 (Adjusted R Squared = .200)
m R Squared = .190 (Adjusted R Squared = .157)
n R Squared = .196 (Adjusted R Squared = .163)
o R Squared = .217 (Adjusted R Squared = .185)
p R Squared = .229 (Adjusted R Squared = .197)
q R Squared = .192 (Adjusted R Squared = .159)
r R Squared = .165 (Adjusted R Squared = .131)
s R Squared = .138 (Adjusted R Squared = .102)

Estimated Marginal Means

1. Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Region1	1023137.526	57358.752	909296.281	1136978.771
Region2	828598.138	41561.866	746109.339	911086.937
Region3	1319799.647	68820.092	1183210.814	1456388.479
Region4	1314689.201	75707.288	1164431.190	1464947.211
Region5	1221099.069	100113.275	1022401.931	1419796.207
Region6	824349.214	163362.179	500120.510	1148577.918
Region7	6680466.025	692180.247	5306679.840	8054252.209
Region8	2154797.512	239119.275	1680211.943	2629383.080
Region9	1150168.513	106169.904	939450.643	1360886.382
Region10	694573.064	64249.527	567055.539	822090.589
Region11	1409597.805	166122.687	1079890.257	1739305.353
Region12	869325.615	97012.323	676783.008	1061868.222
Region13	4853223.788	676706.374	3510148.957	6196298.618
Region14	5570171.185	1311454.449	2967297.132	8173045.238
Region15	11797538.564	1752244.111	8319819.048	15275258.079
Region16	4988937.676	1196779.295	2613662.061	7364213.291

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
Region17	5181410.408	652723.636	3885934.674	6476886.141
Region18	2760015.674	377730.527	2010325.140	3509706.208
Region19	924754.916	124808.082	677045.422	1172464.409
Region20	1585068.311	202095.418	1183964.849	1986171.774

2. species

Estimates

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Region1	1	997978.385	83544.025	832166.620	1163790.149
	2	1053199.273	74155.796	906020.545	1200378.000
	3	860841.724	79104.843	703840.508	1017842.940
	4	985693.750	212996.308	562955.039	1408432.461
	5	1217974.500	134710.693	950611.064	1485337.936
Region2	1	948036.308	60535.585	827889.929	1068182.686
	2	881341.758	53732.920	774696.785	987986.730
	3	725037.724	57318.975	611275.425	838800.023
	4	735184.000	154336.063	428869.636	1041498.364
	5	853390.900	97610.697	659660.686	1047121.114
Region3	1	1612528.423	100237.668	1413584.400	1811472.446
	2	1448967.242	88973.496	1272379.481	1625555.004
	3	1165961.517	94911.454	977588.553	1354334.481
	4	1051526.250	255556.912	544316.522	1558735.978
	5	1320014.800	161628.383	999227.201	1640802.399
Region4	1	1722941.692	110268.990	1504088.272	1941795.113
	2	1485725.758	97877.552	1291465.910	1679985.605
	3	1168943.103	104409.754	961718.645	1376167.562
	4	1017864.750	281131.865	459895.819	1575833.681
	5	1177970.700	177803.403	825080.163	1530861.237
Region5	1	1920144.962	145816.736	1630739.105	2209550.818
	2	1130924.242	129430.633	874040.263	1387808.221
	3	1159925.690	138068.640	885897.658	1433953.721
	4	985544.250	371761.191	247701.195	1723387.305
	5	908956.200	235122.422	442303.278	1375609.122
Region6	1	1509703.769	237939.871	1037458.991	1981948.547
	2	708134.909	211201.464	288958.466	1127311.353
	3	733267.241	225296.734	286115.589	1180418.893
	4	620370.250	606630.022	-583622.420	1824362.920
	5	550269.900	383666.513	-211201.924	1311741.724
Region7	1	9937021.038	1008172.637	7936077.431	11937964.646
	2	6061167.152	894879.604	4285078.853	7837255.450

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
	3	6541355.483	954602.530	4646733.709	8435977.257
	4	6246992.750	2570345.973	1145567.501	11348417.999
	5	4615793.700	1625629.530	1389369.080	7842218.320
Region8	1	3623652.923	348281.406	2932410.741	4314895.105
	2	2018649.182	309143.410	1405085.088	2632213.275
	3	1939260.103	329775.178	1284747.661	2593772.546
	4	1743232.750	887946.843	-19095.936	3505561.436
	5	1449192.600	561586.893	334598.073	2563787.127
Region9	1	1714198.615	154638.322	1407284.353	2021112.878
	2	1441426.515	137260.897	1169001.631	1713851.399
	3	917772.483	146421.483	627166.370	1208378.596
	4	810152.250	394251.910	27671.343	1592633.157
	5	867292.700	249346.801	372408.322	1362177.078
Region10	1	1110107.000	93580.559	924375.494	1295838.506
	2	687199.758	83064.478	522339.762	852059.753
	3	610353.414	88608.077	434490.909	786215.919
	4	559390.250	238584.549	85865.964	1032914.536
	5	505814.900	150894.118	206331.846	805297.954
Region11	1	2284147.385	241960.599	1803922.574	2764372.195
	2	1787259.788	214770.365	1361000.065	2213519.511
	3	1132913.103	229103.818	678205.445	1587620.762
	4	985906.750	616880.908	-238431.090	2210244.590
	5	857762.000	390149.743	83422.760	1632101.240
Region12	1	1449994.846	141300.146	1169553.171	1730436.521
	2	775934.485	125421.593	527007.341	1024861.629
	3	801053.345	133792.043	535513.176	1066593.514
	4	784444.500	360246.100	69455.713	1499433.287
	5	535200.900	227839.639	83002.286	987399.514
Region13	1	9658655.231	985634.670	7702443.248	11614867.213
	2	3344723.758	874874.333	1608340.384	5081107.132
	3	4373912.000	933262.137	2521644.998	6226179.002
	4	4622287.250	2512885.208	-365094.285	9609668.785
	5	2266540.700	1589288.151	-887756.342	5420837.742
Region14	1	14100323.654	1910156.344	10309192.068	17891455.240
	2	3456719.182	1695503.222	91614.626	6821823.738
	3	4501569.138	1808658.569	911882.540	8091255.736
	4	3907738.750	4869962.237	-5757788.217	13573265.717
	5	1884505.200	3080034.558	-4228510.800	7997521.200
Region15	1	22351318.654	2552174.197	17285959.350	27416677.958
	2	7512855.970	2265374.552	3016714.571	12008997.369
	3	13591462.345	2416562.259	8795255.198	18387669.491
	4	10963865.750	6506793.017	-1950317.217	23878048.717

Dependent Variable	species	Mean	Std. Error	95% Confidence Interval	
	5	4568190.100	4115257.239	-3599456.359	12735836.559
Region16	1	13284278.500	1743129.976	9824648.020	16743908.980
	2	2501626.970	1547246.381	-569228.796	5572482.736
	3	3911247.862	1650507.288	635447.778	7187047.946
	4	3848104.250	4444126.881	-4972257.413	12668465.913
	5	1399430.800	2810712.631	-4179055.728	6977917.328
Region17	1	9663135.077	950703.392	7776252.012	11550018.142
	2	3516890.394	843868.445	1842045.125	5191735.663
	3	4708377.517	900186.962	2921755.582	6494999.452
	4	5310747.750	2423827.574	500120.965	10121374.535
	5	2707901.300	1532963.158	-334606.222	5750408.822
Region18	1	5072989.269	550171.119	3981051.893	6164926.646
	2	1886393.030	488345.840	917161.719	2855624.342
	3	2743960.621	520937.311	1710044.262	3777876.980
	4	2759073.750	1402666.636	-24831.245	5542978.745
	5	1337661.700	887124.274	-423034.415	3098357.815
Region19	1	1639267.692	181785.154	1278474.483	2000060.902
	2	679461.606	161357.113	359212.402	999710.810
	3	890697.379	172125.846	549075.223	1232319.536
	4	872146.500	463463.023	-47699.308	1791992.308
	5	542201.400	293119.753	-39560.170	1123962.970
Region20	1	2561523.231	294355.512	1977309.022	3145737.440
	2	1141063.394	261277.418	622500.044	1659626.744
	3	1775778.931	278714.682	1222607.439	2328950.423
	4	1448148.000	750462.249	-41311.825	2937607.825
	5	998828.000	474634.001	56810.894	1940845.106

Univariate Tests

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Region1	Contrast	1130602291150.100	4	2826505727875.525	1.558	.192
	Error	1760256175076 1.740	97	1814697087 70.740		
Region2	Contrast	7858731905 78.698	4	1964682976 44.675	2.062	.092
	Error	9242012750 426.290	97	95278481963. 158		
Region3	Contrast	3358498683 602.875	4	8396246709 0.719	3.214	.016
	Error	2534002206 1515.980	97	2612373408 0.371		
Region4	Contrast	5573496600 408.150	4	1393374150 102.039	4.407	.003
	Error	3066562872 3103.140	97	3161405023 0.032		
Region5	Contrast	1341919444	4	33547986103	6.068	.000

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
		1344.630		36.159		
	Error	5362407662 4689.500	97	55282553221 3.294		
Region6	Contrast	1322203846 1101.780	4	33055096152 75.445	2.246	.070
	Error	1427839935 49632.300	97	14719999335 01.365		
Region7	Contrast	3183098231 10402.700	4	79577455777 600.600	3.011	.022
	Error	2563391228 095911.000	97	26426713691 710.430		
Region8	Contrast	6026654975 6842.400	4	15066637439 210.610	4.777	.001
	Error	3059184429 46264.700	97	31537983808 89.327		
Region9	Contrast	1215291731 8508.300	4	30382293296 27.076	4.887	.001
	Error	6030861257 4924.400	97	62173827396 8.294		
Region10	Contrast	4808863960 385.920	4	12022159900 96.482	5.280	.001
	Error	2208596372 8118.730	97	22769034771 2.564		
Region11	Contrast	2666834165 2087.710	4	66670854130 21.920	4.380	.003
	Error	1476503170 32455.100	97	15221682168 29.434		
Region12	Contrast	9938883262 224.740	4	24847208155 56.187	4.787	.001
	Error	5035357395 1046.000	97	51910900980 4.598		
Region13	Contrast	7376265187 02872.000	4	18440662967 5718.000	7.301	.000
	Error	2450061721 772090.000	97	25258368265 691.650		
Region14	Contrast	2160310924 117537.000	4	54007773102 9384.000	5.693	.000
	Error	9202014490 046860.000	97	94866128763 369.700		
Region15	Contrast	4003175826 269173.000	4	10007939565 67293.000	5.909	.000
	Error	1642728188 0682210.00 0	97	16935342145 0332.100		
Region16	Contrast	2123475831 004042.000	4	53086895775 1010.000	6.720	.000
	Error	7663102330 176800.000	97	79001054950 276.300		
Region17	Contrast	6759131868 02739.000	4	16897829670 0684.800	7.191	.000
	Error	2279476761 653389.000	97	23499760429 416.380		
Region18	Contrast	1818141421 25709.800	4	45453535531 427.400	5.776	.000
	Error	7633797923 91340.000	97	78698947669 21.030		
Region19	Contrast	1647532671 6440.680	4	41188316791 10.171	4.794	.001
	Error	8334161385	97	85919189540		

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
Region20	Contrast	4499.600 3490981620 9001.750	4	7.213 87274540522 50.430	3.874	.006
	Error	2185191117 21106.300	97	22527743476 40.272		

The F tests the effect of species. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

species

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
Region1	1	2	-55220.89	111708.040	.988	-365742.02	255300.24
		3	137136.66	115052.946	.756	-182682.49	456955.81
		4	12284.63	228794.736	1.000	-623708.98	648278.25
		5	-219996.12	158513.643	.637	-660625.42	220633.19
	2	1	55220.89	111708.040	.988	-255300.24	365742.02
		3	192357.55	108428.125	.395	-109046.22	493761.31
		4	67505.52	225536.049	.998	-559429.74	694440.78
		5	-164775.23	153772.732	.821	-592225.95	262675.50
	3	1	-137136.66	115052.946	.756	-456955.81	182682.49
		2	-192357.55	108428.125	.395	-493761.31	109046.22
		4	-124852.03	227211.363	.982	-756444.25	506740.20
		5	-357132.78	156219.548	.158	-791385.05	77119.50
	4	1	-12284.63	228794.736	1.000	-648278.25	623708.98
		2	-67505.52	225536.049	.998	-694440.78	559429.74
		3	124852.03	227211.363	.982	-506740.20	756444.25
		5	-232280.75	252020.630	.888	-932836.69	468275.19
	5	1	219996.12	158513.643	.637	-220633.19	660625.42
		2	164775.23	153772.732	.821	-262675.50	592225.95
		3	357132.78	156219.548	.158	-77119.50	791385.05
		4	232280.75	252020.630	.888	-468275.19	932836.69
Region2	1	2	66694.55	80943.089	.923	-158307.51	291696.62
		3	222998.58	83366.791	.065	-8740.78	454737.95
		4	212852.31	165783.526	.702	-247985.50	673690.11
		5	94645.41	114858.196	.923	-224632.39	413923.20
	2	1	-66694.55	80943.089	.923	-291696.62	158307.51
		3	156304.03	78566.479	.279	-62091.63	374699.70
		4	146157.76	163422.297	.898	-308116.41	600431.92
		5	27950.86	111422.955	.999	-281777.81	337679.52
	3	1	-222998.58	83366.791	.065	-454737.95	8740.78
		2	-156304.03	78566.479	.279	-374699.70	62091.63
		4	-10146.28	164636.221	1.000	-467794.85	447502.30
		5	-128353.18	113195.906	.788	-443010.21	186303.86
	4	1	-212852.31	165783.526	.702	-673690.11	247985.50
		2	-146157.76	163422.297	.898	-600431.92	308116.41
		3	10146.28	164636.221	1.000	-447502.30	467794.85
		5	-118206.90	182612.893	.967	-625826.24	389412.44
	5	1	-94645.41	114858.196	.923	-413923.20	224632.39

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		2	-27950.86	111422.955	.999	-337679.52	281777.81
		3	128353.18	113195.906	.788	-186303.86	443010.21
		4	118206.90	182612.893	.967	-389412.44	625826.24
Region3	1	2	163561.18	134029.374	.740	-209007.82	536130.18
		3	446566.91(*)	138042.654	.014	62841.97	830291.85
		4	561002.17	274512.159	.253	-202074.74	1324079.08
		5	292513.62	190187.602	.541	-236161.57	821188.81
	2	1	-163561.18	134029.374	.740	-536130.18	209007.82
		3	283005.73	130094.071	.198	-78624.09	644635.54
		4	397440.99	270602.325	.585	-354767.53	1149649.52
		5	128952.44	184499.369	.956	-383910.85	641815.73
	3	1	-446566.91(*)	138042.654	.014	-830291.85	-62841.97
		2	-283005.73	130094.071	.198	-644635.54	78624.09
		4	114435.27	272612.398	.993	-643360.77	872231.30
		5	-154053.28	187435.104	.923	-675077.20	366970.63
	4	1	-561002.17	274512.159	.253	-1324079.08	202074.74
		2	-397440.99	270602.325	.585	-1149649.52	354767.53
		3	-114435.27	272612.398	.993	-872231.30	643360.77
		5	-268488.55	302379.016	.901	-1109028.53	572051.43
	5	1	-292513.62	190187.602	.541	-821188.81	236161.57
		2	-128952.44	184499.369	.956	-641815.73	383910.85
		3	154053.28	187435.104	.923	-366970.63	675077.20
		4	268488.55	302379.016	.901	-572051.43	1109028.53
Region4	1	2	237215.93	147442.414	.495	-172638.05	647069.92
		3	553998.59(*)	151857.324	.004	131872.23	976124.95
		4	705076.94	301984.065	.143	-134365.17	1544519.06
		5	544970.99	209220.698	.077	-36611.57	1126553.55
	2	1	-237215.93	147442.414	.495	-647069.92	172638.05
		3	316782.65	143113.283	.183	-81037.40	714602.71
		4	467861.01	297682.953	.519	-359625.07	1295347.08
		5	307755.06	202963.212	.555	-256433.22	871943.33
	3	1	-553998.59(*)	151857.324	.004	-976124.95	-131872.23
		2	-316782.65	143113.283	.183	-714602.71	81037.40
		4	151078.35	299894.185	.987	-682554.41	984711.11
		5	-9027.60	206192.742	1.000	-582193.18	564137.98
	4	1	-705076.94	301984.065	.143	-1544519.06	134365.17
		2	-467861.01	297682.953	.519	-1295347.08	359625.07
		3	-151078.35	299894.185	.987	-984711.11	682554.41
		5	-160105.95	332639.709	.989	-1084763.29	764551.39
	5	1	-544970.99	209220.698	.077	-1126553.55	36611.57
		2	-307755.06	202963.212	.555	-871943.33	256433.22
		3	9027.60	206192.742	1.000	-564137.98	582193.18
		4	160105.95	332639.709	.989	-764551.39	1084763.29
Region5	1	2	789220.72(*)	194973.868	.001	247240.87	1331200.56
		3	760219.27(*)	200812.026	.002	202010.77	1318427.77
		4	934600.71	399335.578	.141	-175454.89	2044656.31

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		5	1011188.76(*)	276667.804	.004	242119.68	1780257.84
	2	1	-789220.72(*)	194973.868	.001	-1331200.56	-247240.87
		3	-29001.45	189249.143	1.000	-555067.95	497065.06
		4	145379.99	393647.903	.996	-948865.26	1239625.24
		5	221968.04	268393.074	.922	-524099.30	968035.39
	3	1	-760219.27(*)	200812.026	.002	-1318427.77	-202010.77
		2	29001.45	189249.143	1.000	-497065.06	555067.95
		4	174381.44	396571.976	.992	-927992.02	1276754.90
		5	250969.49	272663.717	.888	-506969.20	1008908.18
	4	1	-934600.71	399335.578	.141	-2044656.31	175454.89
		2	-145379.99	393647.903	.996	-1239625.24	948865.26
		3	-174381.44	396571.976	.992	-1276754.90	927992.02
		5	76588.05	439873.773	1.000	-1146153.85	1299329.95
	5	1	-1011188.76(*)	276667.804	.004	-1780257.84	-242119.68
		2	-221968.04	268393.074	.922	-968035.39	524099.30
		3	-250969.49	272663.717	.888	-1008908.18	506969.20
		4	-76588.05	439873.773	1.000	-1299329.95	1146153.85
Region6	1	2	801568.86	318153.172	.095	-82819.43	1685957.15
		3	776436.53	327679.722	.133	-134433.25	1687306.31
		4	889333.52	651625.172	.651	-922025.68	2700692.72
		5	959433.87	451459.163	.218	-295512.59	2214380.33
	2	1	-801568.86	318153.172	.095	-1685957.15	82819.43
		3	-25132.33	308811.718	1.000	-883553.66	833288.99
		4	87764.66	642344.177	1.000	-1697795.63	1873324.95
		5	157865.01	437956.678	.996	-1059547.84	1375277.86
	3	1	-776436.53	327679.722	.133	-1687306.31	134433.25
		2	25132.33	308811.718	1.000	-833288.99	883553.66
		4	112896.99	647115.602	1.000	-1685926.70	1911720.68
		5	182997.34	444925.400	.994	-1053786.86	1419781.54
	4	1	-889333.52	651625.172	.651	-2700692.72	922025.68
		2	-87764.66	642344.177	1.000	-1873324.95	1697795.63
		3	-112896.99	647115.602	1.000	-1911720.68	1685926.70
		5	70100.35	717774.322	1.000	-1925137.36	2065338.06
	5	1	-959433.87	451459.163	.218	-2214380.33	295512.59
		2	-157865.01	437956.678	.996	-1375277.86	1059547.84
		3	-182997.34	444925.400	.994	-1419781.54	1053786.86
		4	-70100.35	717774.322	1.000	-2065338.06	1925137.36
Region7	1	2	3875853.89(*)	1348043.609	.039	128621.14	7623086.64
		3	3395665.56	1388408.46	.112	-463771.65	7255102.76

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		1					
		4	3690028.29	2760994.474	.669	-3984863.57	11364920.15
		5	5321227.34(*)	1912873.084	.050	3906.28	10638548.40
	2	1	-3875853.89(*)	1348043.609	.039	-7623086.64	-128621.14
		3	-480188.33	1308463.028	.996	-4117396.71	3157020.05
		4	-185825.60	2721670.062	1.000	-7751405.18	7379753.98
		5	1445373.45	1855661.843	.936	-3712914.30	6603661.20
	3	1	-3395665.56	1388408.461	.112	-7255102.76	463771.65
		2	480188.33	1308463.028	.996	-3157020.05	4117396.71
		4	294362.73	2741887.017	1.000	-7327415.05	7916140.52
		5	1925561.78	1885188.945	.845	-3314804.12	7165927.68
	4	1	-3690028.29	2760994.474	.669	-11364920.15	3984863.57
		2	185825.60	2721670.062	1.000	-7379753.98	7751405.18
		3	-294362.73	2741887.017	1.000	-7916140.52	7327415.05
		5	1631199.05	3041274.370	.983	-6822802.63	10085200.73
	5	1	-5321227.34(*)	1912873.084	.050	-10638548.40	-3906.28
		2	-1445373.45	1855661.843	.936	-6603661.20	3712914.30
		3	-1925561.78	1885188.945	.845	-7165927.68	3314804.12
		4	-1631199.05	3041274.370	.983	-10085200.73	6822802.63
Region8	1	2	1605003.74(*)	465692.587	.007	310491.83	2899515.66
		3	1684392.82(*)	479636.952	.006	351118.97	3017666.67
		4	1880420.17	953807.912	.288	-770933.41	4531773.75
		5	2174460.32(*)	660817.506	.012	337548.68	4011371.97
	2	1	-1605003.74(*)	465692.587	.007	-2899515.66	-310491.83
		3	79389.08	452019.155	1.000	-1177114.03	1335892.18
		4	275416.43	940222.975	.998	-2338174.33	2889007.20
		5	569456.58	641053.419	.901	-1212515.72	2351428.88
	3	1	-1684392.82(*)	479636.952	.006	-3017666.67	-351118.97
		2	-79389.08	452019.155	1.000	-1335892.18	1177114.03
		4	196027.35	947207.086	1.000	-2436977.54	2829032.24
		5	490067.50	651253.796	.943	-1320259.36	2300394.36
	4	1	-1880420.17	953807.912	.288	-4531773.75	770933.41
		2	-275416.43	940222.975	.998	-2889007.20	2338174.33
		3	-196027.35	947207.086	1.00	-2829032.24	2436977.54

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
					0		
		5	294040.15	1050632.873	.999	-2626463.23	3214543.53
	5	1	-2174460.32(*)	660817.506	.012	-4011371.97	-337548.68
		2	-569456.58	641053.419	.901	-2351428.88	1212515.72
		3	-490067.50	651253.796	.943	-2300394.36	1320259.36
		4	-294040.15	1050632.873	.999	-3214543.53	2626463.23
Region9	1	2	272772.10	206769.350	.680	-301996.31	847540.51
		3	796426.13(*)	212960.704	.003	204447.27	1388405.00
		4	904046.37	423494.485	.214	-273165.11	2081257.84
		5	846905.92(*)	293405.586	.038	31309.88	1662501.95
	2	1	-272772.10	206769.350	.680	-847540.51	301996.31
		3	523654.03	200698.292	.077	-34238.32	1081546.38
		4	631274.27	417462.720	.557	-529170.37	1791718.90
		5	574133.82	284630.253	.266	-217068.93	1365336.56
	3	1	-796426.13(*)	212960.704	.003	-1388405.00	-204447.27
		2	-523654.03	200698.292	.077	-1081546.38	34238.32
		4	107620.23	420563.692	.999	-1061444.35	1276684.82
		5	50479.78	289159.261	1.000	-753312.50	854272.07
	4	1	-904046.37	423494.485	.214	-2081257.84	273165.11
		2	-631274.27	417462.720	.557	-1791718.90	529170.37
		3	-107620.23	420563.692	.999	-1276684.82	1061444.35
		5	-57140.45	466485.151	1.000	-1353855.50	1239574.60
	5	1	-846905.92(*)	293405.586	.038	-1662501.95	-31309.88
		2	-574133.82	284630.253	.266	-1365336.56	217068.93
		3	-50479.78	289159.261	1.000	-854272.07	753312.50
		4	57140.45	466485.151	1.000	-1239574.60	1353855.50
Region10	1	2	422907.24(*)	125128.049	.009	75081.76	770732.73
		3	499753.59(*)	128874.793	.002	141513.06	857994.11
		4	550716.75	256280.916	.208	-161681.75	1263115.25
		5	604292.10(*)	177556.627	.008	110727.94	1097856.26
	2	1	-422907.24(*)	125128.049	.009	-770732.73	-75081.76
		3	76846.34	121454.102	.969	-260766.46	414459.15
		4	127809.51	252630.747	.987	-574442.41	830061.43
		5	181384.86	172246.168	.830	-297417.52	660187.23
	3	1	-499753.59(*)	128874.793	.002	-857994.11	-141513.06
		2	-76846.34	121454.102	.969	-414459.15	260766.46
		4	50963.16	254507.325	1.000	-656505.18	758431.51
		5	104538.51	174986.931	.975	-381882.52	590959.54
	4	1	-550716.75	256280.916	.208	-1263115.25	161681.75
		2	-127809.51	252630.747	.987	-830061.43	574442.41
		3	-50963.16	254507.325	1.000	-758431.51	656505.18
		5	53575.35	282297.045	1.000	-731141.65	838292.35

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
	5	1	-604292.10(*)	177556.627	.008	-1097856.26	-110727.94
		2	-181384.86	172246.168	.830	-660187.23	297417.52
		3	-104538.51	174986.931	.975	-590959.54	381882.52
		4	-53575.35	282297.045	1.000	-838292.35	731141.65
Region11	1	2	496887.60	323529.351	.542	-402445.16	1396220.36
		3	1151234.28(*)	333216.882	.007	224972.54	2077496.02
		4	1298240.63	662636.390	.294	-543727.06	3140208.33
		5	1426385.38(*)	459087.958	.020	150232.73	2702538.04
	2	1	-496887.60	323529.351	.542	-1396220.36	402445.16
		3	654346.68	314030.044	.236	-218580.32	1527273.69
		4	801353.04	653198.564	.736	-1014379.80	2617085.88
		5	929497.79	445357.307	.234	-308487.01	2167482.58
	3	1	-1151234.28(*)	333216.882	.007	-2077496.02	-224972.54
		2	-654346.68	314030.044	.236	-1527273.69	218580.32
		4	147006.35	658050.616	.999	-1682214.01	1976226.72
		5	275151.10	452443.788	.973	-982532.38	1532834.59
	4	1	-1298240.63	662636.390	.294	-3140208.33	543727.06
		2	-801353.04	653198.564	.736	-2617085.88	1014379.80
		3	-147006.35	658050.616	.999	-1976226.72	1682214.01
		5	128144.75	729903.333	1.000	-1900808.66	2157098.16
	5	1	-1426385.38(*)	459087.958	.020	-2702538.04	-150232.73
		2	-929497.79	445357.307	.234	-2167482.58	308487.01
		3	-275151.10	452443.788	.973	-1532834.59	982532.38
		4	-128144.75	729903.333	1.000	-2157098.16	1900808.66
Region12	1	2	674060.36(*)	188934.664	.005	148868.03	1199252.69
		3	648941.50(*)	194591.988	.010	108023.19	1189859.81
		4	665550.35	386966.386	.427	-410121.91	1741222.61
		5	914793.95(*)	268098.176	.008	169546.34	1660041.55
	2	1	-674060.36(*)	188934.664	.005	-1199252.69	-148868.03
		3	-25118.86	183387.259	1.000	-534890.75	484653.03
		4	-8510.02	381454.884	1.000	-1068861.64	1051841.61
		5	240733.58	260079.751	.886	-482224.75	963691.92
	3	1	-648941.50(*)	194591.988	.010	-1189859.81	-108023.19
		2	25118.86	183387.259	1.000	-484653.03	534890.75
		4	16608.84	384288.385	1.000	-1051619.23	1084836.92
		5	265852.44	264218.114	.852	-468609.53	1000314.42
	4	1	-665550.35	386966.386	.427	-1741222.61	410121.91
		2	8510.02	381454.884	1.000	-1051841.61	1068861.64
		3	-16608.84	384288.385	1.000	-1084836.92	1051619.23
		5	249243.60	426248.934	.977	-935624.57	1434111.77
	5	1	-914793.95(*)	268098.176	.008	-1660041.55	-169546.34
		2	-240733.58	260079.751	.886	-963691.92	482224.75

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		3	-265852.44	264218.114	.852	-1000314.42	468609.53
		4	-249243.60	426248.934	.977	-1434111.77	935624.57
Region13	1	2	6313931.47(*)	1317907.736	.000	2650469.10	9977393.84
		3	5284743.23(*)	1357370.222	.002	1511584.77	9057901.69
		4	5036367.98	2699271.711	.343	-2466949.64	12539685.60
		5	7392114.53(*)	1870110.299	.001	2193663.59	12590565.47
	2	1	-6313931.47(*)	1317907.736	.000	-9977393.84	-2650469.10
		3	-1029188.24	1279211.990	.929	-4585085.87	2526709.38
		4	-1277563.49	2660826.406	.989	-8674012.53	6118885.55
		5	1078183.06	1814178.030	.976	-3964789.80	6121155.92
	3	1	-5284743.23(*)	1357370.222	.002	-9057901.69	-1511584.77
		2	1029188.24	1279211.990	.929	-2526709.38	4585085.87
		4	-248375.25	2680591.405	1.000	-7699766.17	7203015.67
		5	2107371.30	1843045.046	.783	-3015844.83	7230587.43
	4	1	-5036367.98	2699271.711	.343	-12539685.60	2466949.64
		2	1277563.49	2660826.406	.989	-6118885.55	8674012.53
		3	248375.25	2680591.405	1.000	-7203015.67	7699766.17
		5	2355746.55	2973285.875	.932	-5909263.68	10620756.78
	5	1	-7392114.53(*)	1870110.299	.001	-12590565.47	-2193663.59
		2	-1078183.06	1814178.030	.976	-6121155.92	3964789.80
		3	-2107371.30	1843045.046	.783	-7230587.43	3015844.83
		4	-2355746.55	2973285.875	.932	-10620756.78	5909263.68
Region14	1	2	10643604.47(*)	2554100.318	.001	3543827.95	17743380.99
		3	9598754.52(*)	2630578.469	.004	2286387.37	16911121.66
		4	10192584.90	5231178.591	.299	-4348816.89	24733986.70
		5	12215818.45(*)	3624266.841	.009	2141239.77	22290397.14
	2	1	-10643604.47(*)	2554100.318	.001	-17743380.99	-3543827.95
		3	-1044849.96	2479108.105	.993	-7936166.40	5846466.49
		4	-451019.57	5156671.733	1.000	-14785310.45	13883271.31
		5	1572213.98	3515870.312	.992	-8201048.77	11345476.73

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
	3	1	-9598754.52(*)	2630578.469	.004	-16911121.66	-2286387.37
		2	1044849.96	2479108.105	.993	-5846466.49	7936166.40
		4	593830.39	5194976.228	1.000	-13846937.66	15034598.43
		5	2617063.94	3571814.482	.948	-7311709.97	12545837.85
	4	1	-10192584.90	5231178.591	.299	-24733986.70	4348816.89
		2	451019.57	5156671.733	1.000	-13883271.31	14785310.45
		3	-593830.39	5194976.228	1.000	-15034598.43	13846937.66
		5	2023233.55	5762217.027	.997	-13994325.70	18040792.80
	5	1	-12215818.45(*)	3624266.841	.009	-22290397.14	-2141239.77
		2	-1572213.98	3515870.312	.992	-11345476.73	8201048.77
		3	-2617063.94	3571814.482	.948	-12545837.85	7311709.97
		4	-2023233.55	5762217.027	.997	-18040792.80	13994325.70
Region15	1	2	14838462.68(*)	3412552.563	.000	5352398.10	24324527.27
		3	8759856.31	3514735.593	.101	-1010252.20	18529964.81
		4	11387452.90	6989416.892	.483	-8041422.93	30816328.74
		5	17783128.55(*)	4842410.069	.004	4322408.52	31243848.59
	2	1	-14838462.68(*)	3412552.563	.000	-24324527.27	-5352398.10
		3	-6078606.38	3312354.905	.360	-15286145.88	3128933.13
		4	-3451009.78	6889867.722	.987	-22603163.18	15701143.62
		5	2944665.87	4697580.655	.970	-10113463.68	16002795.42
	3	1	-8759856.31	3514735.593	.101	-18529964.81	1010252.20
		2	6078606.38	3312354.905	.360	-3128933.13	15286145.88
		4	2627596.59	6941046.644	.996	-16666821.74	21922014.93
		5	9023272.24	4772328.080	.329	-4242636.93	22289181.42
	4	1	-11387452.90	6989416.892	.483	-30816328.74	8041422.93
		2	3451009.78	6889867.722	.987	-15701143.62	22603163.18
		3	-2627596.59	6941046.644	.996	-21922014.93	16666821.74
		5	6395675.65	7698941.324	.920	-15005505.22	27796856.52
	5	1	-17783128.55(*)	4842410.069	.004	-31243848.59	-4322408.52
		2	-2944665.87	4697580.65	.970	-	10113463.68

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		5				16002795.42	
		3	-9023272.24	4772328.080	.329	-22289181.42	4242636.93
		4	-6395675.65	7698941.324	.920	-27796856.52	15005505.22
Region16	1	2	10782651.53(*)	2330766.714	.000	4303688.01	17261615.05
		3	9373030.64(*)	2400557.523	.002	2700065.67	16045995.60
		4	9436174.25	4773758.043	.285	-3833709.88	22706058.38
		5	11884847.70(*)	3307356.589	.005	2691202.29	21078493.11
	2	1	-10782651.53(*)	2330766.714	.000	-17261615.05	-4303688.01
		3	-1409620.89	2262331.910	.971	-7698352.34	4879110.55
		4	-1346477.28	4705766.154	.999	-14427360.53	11734405.97
		5	1102196.17	3208438.383	.997	-7816480.73	10020873.07
	3	1	-9373030.64(*)	2400557.523	.002	-16045995.60	-2700065.67
		2	1409620.89	2262331.910	.971	-4879110.55	7698352.34
		4	63143.61	4740721.258	1.000	-13114906.31	13241193.53
		5	2511817.06	3259490.728	.938	-6548772.91	11572407.04
	4	1	-9436174.25	4773758.043	.285	-22706058.38	3833709.88
		2	1346477.28	4705766.154	.999	-11734405.97	14427360.53
		3	-63143.61	4740721.258	1.000	-13241193.53	13114906.31
		5	2448673.45	5258361.839	.990	-12168291.15	17065638.05
	5	1	-11884847.70(*)	3307356.589	.005	-21078493.11	-2691202.29
		2	-1102196.17	3208438.383	.997	-10020873.07	7816480.73
		3	-2511817.06	3259490.728	.938	-11572407.04	6548772.91
		4	-2448673.45	5258361.839	.990	-17065638.05	12168291.15
Region17	1	2	6146244.68(*)	1271200.571	.000	2612616.85	9679872.51
		3	4954757.56(*)	1309264.491	.002	1315321.31	8594193.81
		4	4352387.33	2603608.467	.456	-2885009.78	11589784.44
		5	6955233.78(*)	1803832.859	.002	1941017.97	11969449.58
	2	1	-6146244.68(*)	1271200.571	.000	-9679872.51	-2612616.85
		3	-1191487.12	1233876.217	.870	-4621362.35	2238388.10
		4	-1793857.36	2566525.679	.956	-8928173.35	5340458.64

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
		5	808989.09	1749882.852	.990	-4055258.84	5673237.03
	3	1	-4954757.56(*)	1309264.491	.002	-8594193.81	-1315321.31
		2	1191487.12	1233876.217	.870	-2238388.10	4621362.35
		4	-602370.23	2585590.198	.999	-7789680.95	6584940.48
		5	2000476.22	1777726.810	.793	-2941171.13	6942123.57
	4	1	-4352387.33	2603608.467	.456	-11589784.44	2885009.78
		2	1793857.36	2566525.679	.956	-5340458.64	8928173.35
		3	602370.23	2585590.198	.999	-6584940.48	7789680.95
		5	2602846.45	2867911.461	.893	-5369248.58	10574941.48
	5	1	-6955233.78(*)	1803832.859	.002	-11969449.58	-1941017.97
		2	-808989.09	1749882.852	.990	-5673237.03	4055258.84
		3	-2000476.22	1777726.810	.793	-6942123.57	2941171.13
		4	-2602846.45	2867911.461	.893	-10574941.48	5369248.58
Region18	1	2	3186596.24(*)	735642.522	.000	1141689.28	5231503.19
		3	2329028.65(*)	757670.075	.022	222890.46	4435166.83
		4	2313915.52	1506705.662	.542	-1874359.08	6502190.12
		5	3735327.57(*)	1043876.303	.005	833605.81	6637049.33
	2	1	-3186596.24(*)	735642.522	.000	-5231503.19	-1141689.28
		3	-857567.59	714042.955	.751	-2842433.01	1127297.83
		4	-872680.72	1485245.889	.977	-5001302.38	3255940.94
		5	548731.33	1012655.487	.983	-2266204.17	3363666.83
	3	1	-2329028.65(*)	757670.075	.022	-4435166.83	-222890.46
		2	857567.59	714042.955	.751	-1127297.83	2842433.01
		4	-15113.13	1496278.508	1.000	-4174402.78	4144176.52
		5	1406298.92	1028768.758	.650	-1453427.55	4266025.39
	4	1	-2313915.52	1506705.662	.542	-6502190.12	1874359.08
		2	872680.72	1485245.889	.977	-3255940.94	5001302.38
		3	15113.13	1496278.508	1.000	-4144176.52	4174402.78
		5	1421412.05	1659657.546	.912	-3192031.51	6034855.61
	5	1	-3735327.57(*)	1043876.303	.005	-6637049.33	-833605.81
		2	-548731.33	1012655.487	.983	-3363666.83	2266204.17
		3	-1406298.92	1028768.75	.650	-4266025.39	1453427.55

Dependent Variable	(I) species	(J) species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower and Upper	
				8			
		4	-1421412.05	1659657.546	.912	-6034855.61	3192031.51
Region19	1	2	959806.09(*)	243067.810	.001	284136.80	1635475.37
		3	748570.31(*)	250346.058	.028	52669.27	1444471.35
		4	767121.19	497839.147	.539	-616750.33	2150992.71
		5	1097066.29(*)	344913.078	.017	138291.98	2055840.60
	2	1	-959806.09(*)	243067.810	.001	-1635475.37	-284136.80
		3	-211235.77	235930.975	.898	-867066.39	444594.85
		4	-192684.89	490748.502	.995	-1556846.15	1171476.36
		5	137260.21	334597.232	.994	-792838.57	1067358.98
	3	1	-748570.31(*)	250346.058	.028	-1444471.35	-52669.27
		2	211235.77	235930.975	.898	-444594.85	867066.39
		4	18550.88	494393.852	1.000	-1355743.56	1392845.32
		5	348495.98	339921.309	.843	-596402.43	1293394.39
	4	1	-767121.19	497839.147	.539	-2150992.71	616750.33
		2	192684.89	490748.502	.995	-1171476.36	1556846.15
		3	-18550.88	494393.852	1.000	-1392845.32	1355743.56
		5	329945.10	548376.844	.975	-1194408.90	1854299.10
	5	1	-1097066.29(*)	344913.078	.017	-2055840.60	-138291.98
		2	-137260.21	334597.232	.994	-1067358.98	792838.57
		3	-348495.98	339921.309	.843	-1293394.39	596402.43
		4	-329945.10	548376.844	.975	-1854299.10	1194408.90
Region20	1	2	1420459.84(*)	393587.419	.004	326382.72	2514536.96
		3	785744.30	405372.719	.304	-341093.08	1912581.68
		4	1113375.23	806125.768	.641	-1127457.97	3354208.44
		5	1562695.23(*)	558500.315	.048	10200.45	3115190.02
	2	1	-1420459.84(*)	393587.419	.004	-2514536.96	-326382.72
		3	-634715.54	382031.103	.463	-1696668.91	427237.84
		4	-307084.61	794644.245	.995	-2516001.97	1901832.76
		5	142235.39	541796.386	.999	-1363826.54	1648297.33
	3	1	-785744.30	405372.719	.304	-1912581.68	341093.08
		2	634715.54	382031.103	.463	-427237.84	1696668.91
		4	327630.93	800546.976	.994	-1897694.59	2552956.45
		5	776950.93	550417.395	.622	-753075.31	2306977.17
	4	1	-1113375.23	806125.768	.641	-3354208.44	1127457.97
		2	307084.61	794644.245	.995	-1901832.76	2516001.97
		3	-327630.93	800546.976	.994	-2552956.45	1897694.59
		5	449320.00	887958.908	.987	-2018989.39	2917629.39
	5	1	-1562695.23(*)	558500.315	.048	-3115190.02	-10200.45
		2	-142235.39	541796.386	.999	-1648297.33	1363826.54
		3	-776950.93	550417.395	.622	-2306977.17	753075.31
		4	-449320.00	887958.908	.987	-2917629.39	2018989.39

Based on observed means.
* The mean difference is significant at the .05 level.